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VOLUME 25 NUMBER 6

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Phytopathology

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The American Phytopathological Society

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PATHOLOGICO-ANATOMICAL CHANGES IN THE TOMATO INCIDENT TO DEVELOPMENT OF WOODINESS OF THE FRUIT[1]

P. V. MICHAILOWA

(Accepted for publication May 23, 1934)

Woodiness of tomato fruit has been known in the Crimea and Australia for many years. In August, 1933, it was simultaneously described by Samuel, Bald, and Eardley (6), on the one hand, and by Rischkow, Karatschewsky and Michailowa (4) on the other. This disease, which was described (4) as "fruit woodiness," is known in the Crimea under the name of *stowboor*, while in Australia it is called big bud.

In our preliminary report (4) we described the characteristic anatomical features of plants affected with this disease; some of them also are mentioned in the description given by the Australian authors. In this paper we present a more detailed analyses of histological changes observed in *stowboor*, which form the most interesting features of this disease.

ANATOMY OF THE TOMATO PLANT

Before describing the anomalies in the structure of the stalk, let us consider briefly the structure of a normal tomato stalk. If we examine a cross section of a young tomato stalk from the periphery to the center, we shall note the following: an epidermis of a single layer of cells and assimilating tissue, 3 or 4 cells deep, rich in chloroplasts that lend their green color to the stalk. Next to the assimilating tissue lies the collenchyma, comprising 6 or 7 layers of thick-angle cells. Then we note the cortical parenchyma, a single-cell thickness of endodermis with a great many starch grains. Beyond these structures lie the bicollateral fibrovascular bundles.

The xylem consists of vessels, tracheids, and a little libriform. During the growth of the tomato the wood tissue gains more and more in mass, forming a closed ring. In the phloem there are sieve tubes, satellite cells, phloem parenchyma, and, in older stalks, also, thick bast.

The center of the stalk consists of the big pith-cells. The parenchymatous cells of the pith adjoining the inner phloem contain a few grains of starch.

[1] This investigation was conducted in the Laboratory for Virus Diseases, Ukrainian Institute of Plant Protection (U. S. S. R., Kharkow, Sozselchos House).

Having considered the structure of a normal stalk we may analyze the changes occurring as a result of the woodiness of fruits. If the plant be attacked by the disease at an older age, the part of the stalk that had developed before the plant became infected in no way differs from a healthy plant. Below are recorded the results of an investigation of both a normal and a diseased plant.

A part of the stalk measuring 28 cm. from the top was investigated. Twenty-eight cm. below these the cuts of the normal and the diseased stems were quite identical, for, during the development of these parts of the stalk, the tomato plant had evidently been healthy. The structure of the diseased stalk, as described below, is typical for all stalks characterized by woodiness of fruit.

Normal Plant	*Diseased Plant*
(1) A cross section made 0.5 cm. below the top. The fibrovascular bundles are hardly visible. The xylem consists of a small number of vessels. They lie in the bundle in 1 or rarely 2 rows along the radius. The inner and exterior phloem occupies but little space in the bundles. Starch is found only in the cells of the bundle sheath (endodermis).	(1) In a cross section made 0.5 cm. from the top, the bundles appear to be perfectly developed. The vessels are arranged in 2–3 rows, seldom in 1 row, along the radius. Their number is nearly twice as great as in the normal plant. The inner phloem occupies enormous areas in the bundles. The cells of the sieve tubes are filled with a yellowish substance of unknown nature. Numerous starch grains are present in the cells of the pith, collenchyma, and cortical parenchyma.
(2) At 3 cm. below the top, the activity of the interfascicular cambium is evident, its cells are just beginning to divide, the space between the bundles is occupied by parenchymatous cells. The phloem occupies little space, small groups being visible in the neighborhood of the xylem. Starch only is contained in the endodermis. The fibrovascular bundle looks like that belonging to a diseased plant from 0.5 cm. from the top upwards.	(2) At 3 cm. from the top, the fibrovascular bundles are better developed than those of a healthy plant. The ring of wood tissue is plainly visible. The inner phloem is very broad, forming nearly continuous rings; much starch is present.
(3) At 5 cm. from the top, the same as in the foregoing section. Bundles are very poorly developed; inner phloem occupies only a small part of the bundles. Starch present in the bundle sheath.	(3) 5 cm. The fibrovascular bundles are thicker than in the norm. The ring of xylem is distinctly visible. A very broad inner phloem. Much starch.
(4) At 8 cm. interfascicular xylem with slightly thickened walls has appeared. The inner phloem occupies but little space in the bundles. A few starch grains are met with in the parenchyma, the pith, and also in the bundle sheath.	(4) 8 cm. Bast is present in separate areas in the exterior phloem. The mechanical elements of the interfascicular xylem have thicker walls than in the healthy plant. The inner phloem occupies much greater expanses than in the normal or healthy plant. Much starch in all tissues.
(5) At 10 cm. the differences between the healthy and the diseased stalk are, on the whole, the same as in the former sections. The structural make up recalls that shown in the cross section made at 8 cm.	
(6) At 14 cm. from the top, bast cells are observed here and there. The number of vessels in a bundle is smaller than that in a diseased plant. The quantity and position of starch grains are the same as in the preceding sections.	(6) 14 cm. Bast in the shape of a solid ring, especially distinctly visible near the great fibrovascular bundles. The medullary cells are crowded with starch grains.

(7) At 18 cm. each fibrovascular bundle contains from 63 to 94 vessels. The bast forms a solid ring, although it is not seen in such strong relief. The interfascicular xylem is 0.11 to 0.099 mm. broad. The inner phloem occupies much smaller areas and is present in much smaller quantities than in a diseased stalk. Starch grains are as numerous as in the section made at 8 cm.

(8) At 23 cm. the structure reminds one of that of the foregoing section, only the bast cells are more numerous and more plainly visible.

(9) At 28 cm. the width of the vessels is the same in healthy as in diseased plants. The xylem measures 0.22 mm. in the broadest and 0.099 mm. in the narrowest places. The exterior phloem has one layer of thick bast, which forms a solid ring. In some places bast also is contained in the inner phloem.

(7) At 18 cm. there are 80 to 130 vessels per bundle. The solid bast ring plainly divides the cortical parenchyma from the exterior phloem. The interfascicular xylem is 0.099 mm. to 0.154 mm. broad. The inner phloem forms an almost solid ring inward from the xylem. Very numerous starch grains.

(8) At 23 cm. the structure is markedly like that of the section made at 18 cm. In the inner phloem some few small areas of bast have just appeared.

(9) At 28 cm. the cross sections of vessels look like those of healthy stalks. The interfascicular xylem is better developed than in the healthy plant. The maximum breadth of xylem is 0.264 mm., the minimum, 0.132 mm. In the structure and position of the bast cells the exterior phloem in no way differs from that of a healthy tomato plant. As to the inner phloem, its development is much stronger than the inner phloem of a normal plant or exterior phloem of a diseased plant. The parenchyma of the pith and cortex contains many starch grains.

In summing up the results of the study of the stalk, the following facts should be noted: (1) Under the influence of the virosis the plant develops much more rapidly than a corresponding healthy one. The development of the tissues of healthy tomato plants seems to lag in comparison with that of the diseased ones. For instance, the fibrovascular bundles of a tomato suffering from woodiness of fruit appear to be quite developed at a distance of about 0.5 cm. from the top of the stalk, if the plant be infected; whereas such development occurs only at a distance of 3 cm. from the top if the plant be healthy. The cambium of the diseased plant begins to form xylem and phloem at a distance of 3 cm. from the top, whereas in a healthy individual, this development would occur only at a distance of 8 cm. from the top; bast appears at a distance of 8 cm. in diseased and of 14 cm. in healthy plants.

Moreover, it is observed that xylem cells of a diseased individual earlier acquire a woody consistency than do those of a normal plant. (2) The interfascicular xylem begins to form earlier and develops at a greater rate when the plant is suffering from the disease. At a distance of 28 cm. from the top in a normal plant the xylem measures only about 0.165 mm. in diameter, while in a diseased plant this tissue attains a diameter of 0.198 mm. The number of vessels is twice as great in a diseased plant as in a normal one. (3) The structure of the exterior phloem shows no great difference except that the formation of bast begins earlier and the sieve tubes of a diseased stalk show peculiar contents. As long as the interfascicular xylem has developed no partitions between the bundles, the inner phloem

of a diseased tomato plant occupies more space than the rest of the bundle and is, of course, much more strongly developed than it would be in a healthy plant. The cells of the sieve tubes are filled with a yellowish substance of unknown nature. (4) Of great interest is the difference in the storing of starch grains. In a healthy stalk, starch is found in the starch-bearing layer (bundle sheath) and a little of it is contained in the pith cells surrounding the inner phloem. In the stalk of a tomato plant attacked by this disease, starch is found in the parenchymatous cells of the medulla, the cortex, and the collenchyma. Where starch is found, it is present in such great quantities that the cells are gorged with it. Elements of wood tissue are often observed in the region of the inner phloem and the medulla of diseased plants. The cells of mechanical tissue give a positive reaction to the lignin test and stain like lignin. These elements occupy different spaces. In the inner phloem there is not much of the same, but in the medulla the wood elements form $\frac{1}{4} - \frac{2}{3}$ of the medullar space (Fig. 1, B).

THE LEAF BLADE

A cross section of a healthy leaf shows the upper epidermis, consisting of cells of different sizes and shapes; palisade parenchyma formed of one layer, the ends of the cells of which adjoining the spongy parenchyma are sharp; spongy parenchyma with wide intercellular passages; and lower epidermis of the same structure as the upper one (Fig. 2, A).

The cross section of a leaf of a tomato plant infected by this disease shows a different structure. The upper epidermis is formed of a compact layer of cells of nearly uniform size. Beneath them is the palisade parenchyma with cells that are short in comparison with those of a healthy palisade parenchyma.

The spongy parenchyma consists of 3 layers. Its cells lie close to each other with hardly any intercellular spaces between them. The lower and upper epidermises are structurally the same (Fig. 2, B).

Carbon assimilation is much more intense in the leaves of a normal plant than in a diseased one. The test with chlorohydrate iodide, used to determine the presence of starch, shows that sections of a healthy leaf contain more and larger starch grains.

PETIOLE

Cross sections of the petioles of healthy and diseased tomato leaves show a difference in xylem structure. The entire section through a petiole of a healthy leaf has 75 to 79 vessels, while that of a diseased plant shows as many as 123 to 151. The width of the vessels varies. A vessel of a diseased plant measures 0.036 to 0.0135 mm.; that of a healthy plant 0.0505 to 0.0198 mm. The relative values of the inner and outer phloem are the

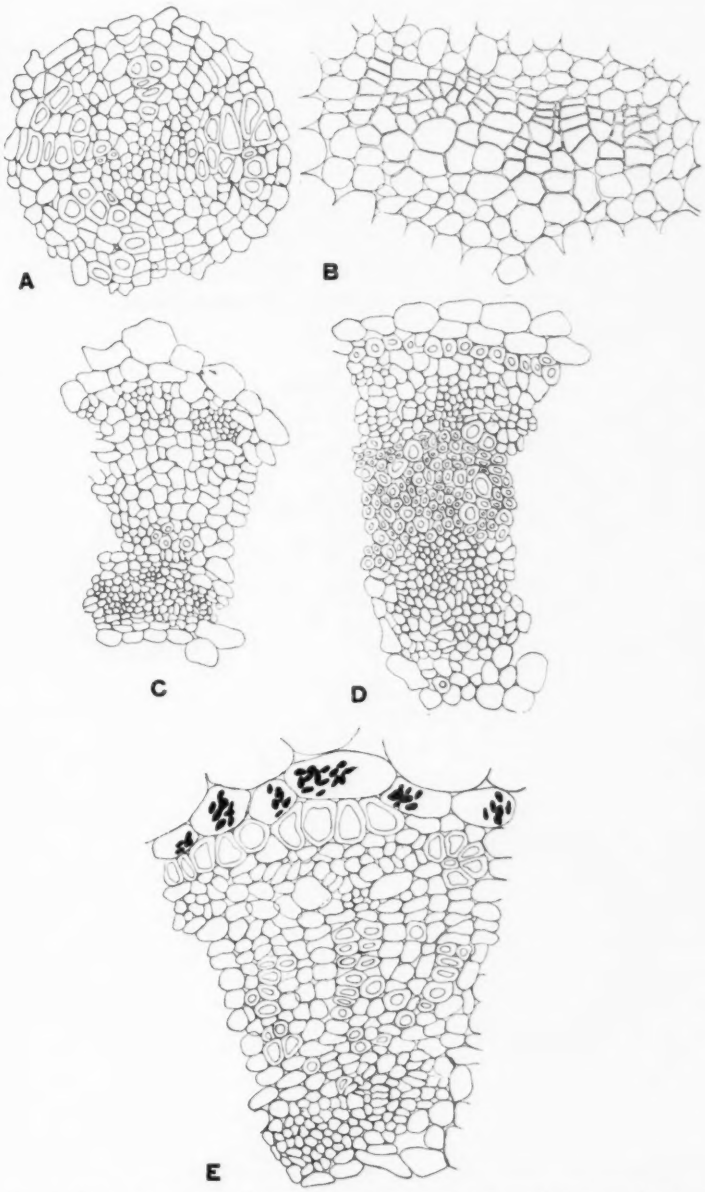

FIG. 1. A. Cross section of fibrovascular bundle of a normal tomato fruit. B. Cross section of stalk of a diseased plant, showing wood tissue of the pith. C and D. Fibrovascular bundles of fruit stalk when tomato fruit was just showing. C. Normal. D. *Stowboor*. E. Cross section through the leaf trace from sepal on the peduncle of a *Stowboor* tomato plant.

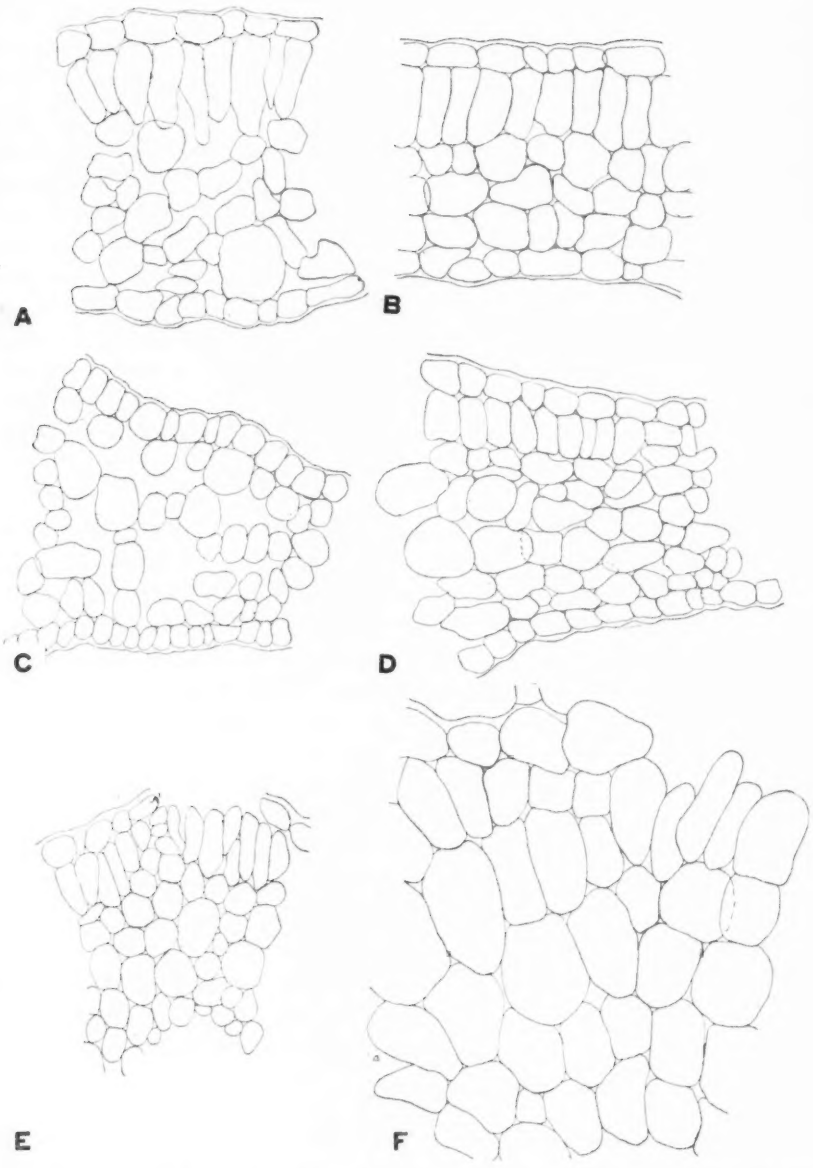

FIG. 2. A and B. Cross sections of tomato leaf. C and D. Cross sections of tomato petal. E and F. Cross sections of tomato sepal. A, C, and E. Healthy. B, D, F. *Stowboor.*

same as for those of the stalk. The inner phloem of the diseased plant is much better developed and the sieve tubes and companion cells are filled with a yellow substance.

PEDUNCLE AND PEDICEL

Schwarz (7) has studied the structure of both the peduncle and the pedicel of the tomato. He performed his anatomical investigation with a view to determining what connection, if any, existed between the development of the tissues of a fruit and that of the tissues of its pedicel.

Let us first consider the anatomical structure of the peduncle and then that of the pedicel. It must be noted that material serving for these inves-

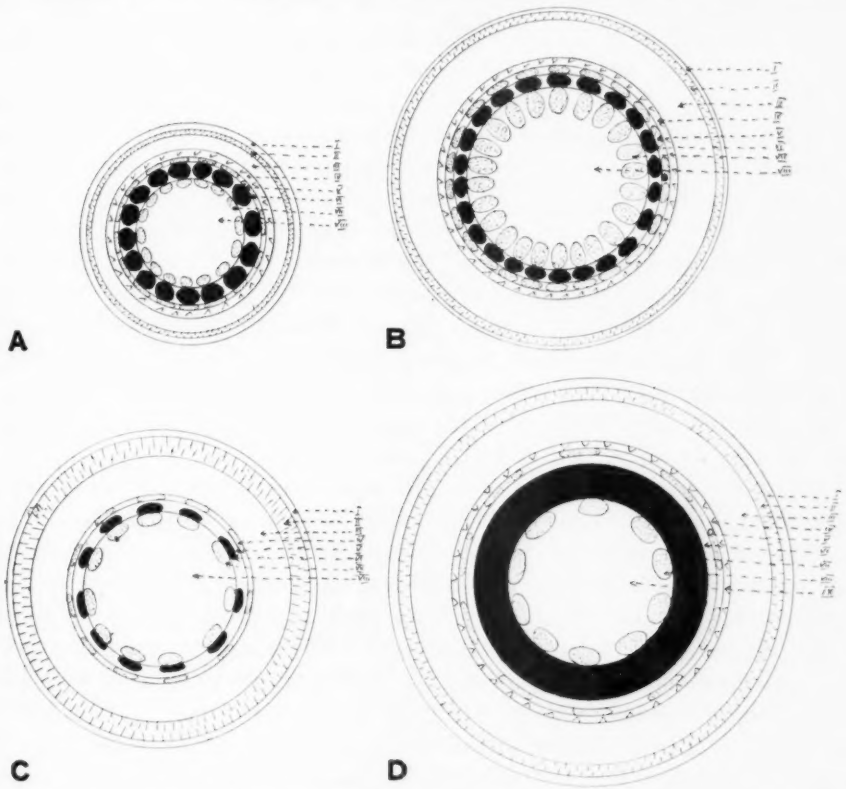

FIG. 3. A and B. Diagrams of tomato peduncle. C and D. Diagrams of tomato fruit stalk when fruit was just beginning to form. A and C. Normal. B and D. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, collenchyma; IV, cortical parenchyma; V, outer phloem; VI, xylem; VII, inner phloem; VIII, pith. In both C and D: I, epidemis; II, assimilating tissue; III, cortical parenchyma. In C: IV, outer phloem; V, cambium; VI, xylem; VII, inner phloem; VIII, pith. In D: IV, bast; V, outer phloem; VI, cambium; VII, xylem; VIII, inner phloem; IX, pith.

tigations was always taken from fruits and flowers of the same age, and the sections were made at the same distance from the top in diseased as in healthy plants. The peduncles were those of flowers whose corollas were open.

The section through a healthy peduncle shows epidermis, 2 layers of assimilating tissue, 3 layers of collenchyma, and nearly as many of cortex parenchyma. The fibrovascular bundles were of bicollateral type, the inner and outer phloems were nearly equal to each other in volume and development. The xylem consisted mainly of vessels and libriform cells. There is considerably more of it than there is of the phloem. The center of the section is occupied by medullary parenchyma. The diameter of the peduncle is about 1.44 mm. (Fig. 3, A).

The cross section of a diseased peduncle shows the same tissues as those of a healthy plant, with this difference, however, that the development of these tissues is richer. The collenchyma consists of 4 to 5 layers, its cells being much bigger; therefore, if the collenchyma of a healthy plant does not exceed 0.066 mm. in breadth, it measures about 0.198 mm. in the peduncle of a diseased plant. The same might be said of the pith, its diameter being 0.462 in healthy and 0.671 in diseased peduncles. The inner phloem requires special attention. As is shown in figure 3, B the area occupied by the inner phloem nearly equals that of the xylem and the outer phloem combined, so that the inner phloem is nearly twice as wide as the outer. The areas of the inner phloem proliferate so much that they form an almost solid ring. The whole peduncle measures 1.793 mm. in breadth.

During the growth of the fruit the difference becomes greater and greater, as is shown by figures 4, A, B, and 3, C, D.

While the fibrovascular bundles are plainly visible in a healthy fruit stalk when the fruit is just starting on its growth (Fig. 3, C and 1, C), in a diseased fruit stalk there is a solid ring of xylem (3, D and 1, D). In the outer phloem of a diseased fruit stalk, bast has formed and surrounds the whole fruit stalk like a ring. The fruit stalk of a diseased plant is about 1 mm. thicker than that of a healthy one, while the fruit itself measures 4 cm. in diameter. The diameter of a healthy fruit stalk is about 2.739 mm., while in a diseased plant it is approximately 3.74 mm. The thickening is due to the development of all the tissues of the fruit stalk (Fig. 4, A and B) but especially to that of the medulla, which has bigger cells in a diseased plant, measuring 1.628 mm. instead of 1.265 mm. in the pedicel. The inner phloem of the pedicel of a diseased plant has a radius of 0.209 mm. instead of 0.099 mm., as in a normal plant. The xylem of the former attains a radius of 0.385 mm. instead of 0.253 mm., and the outer phloem, which measures 0.044 mm. in the pedicel of a healthy plant, attains a radius of 0.099 mm. in that of a diseased one.

In summing up we note that the phenomena observed in both the peduncle and pedicel are the same as those that obtain in the stalk. We see that the tissues of a healthy tomato plant are not growing so fast nor is the development of the tissues so vigorous as that of a diseased plant. The hypertrophy of the bundles is plainly visible in each section, a fact that deserves comparison with the observations made by Schwarz (7), who studied the anatomical structure and solidity of the peduncles of heavy

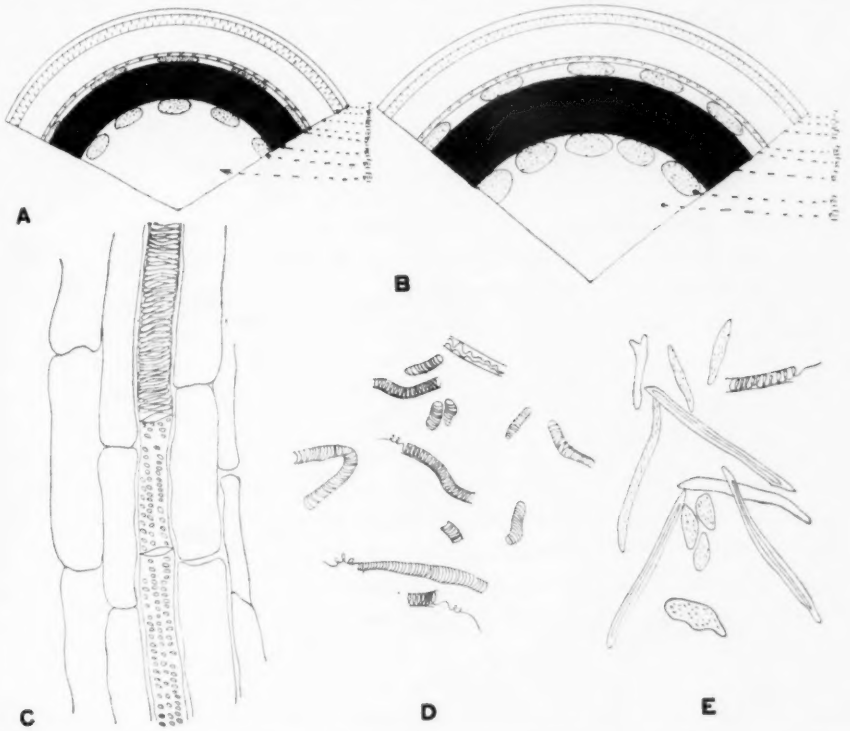

FIG. 4. A and B. Diagrams of old fruit stalks of tomato. A. Normal. B. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, cortical parenchyma; IV, bast; V, outer phloem; VI, xylem; VII, inner phloem; VIII, pith. C. Spiral vessel changed to porous one. D and E. Macerated fibrovascular bundles of tomato fruit. D. Normal. E. *Stowboor*.

fruits, including the tomato. He proved that, contrary to the prevailing opinion of the great practical perfection of the development of mechanical tissue in the pedicel, this tissue shows a development several hundred times greater than that necessary to sustain these heavy fruits. Their firmness and strength are far in excess of the safety quotient required by the rules of engineering. Even the peduncles of the flowers are sufficiently strong, according to Schwarz (7), to sustain the future fruit, so that the develop-

ment of their mechanical tissues, which is correlated to the growth of the fruit, may hardly be considered necessary. We found that, owing to the virus, the pedicel becomes even stronger, although the fruits of plants suffering from this disease generally are smaller than normal ones. This fact proves that solid mechanical structures "perfectly answering the demands of engineering" may be the result of a pathological agent, and not a kind

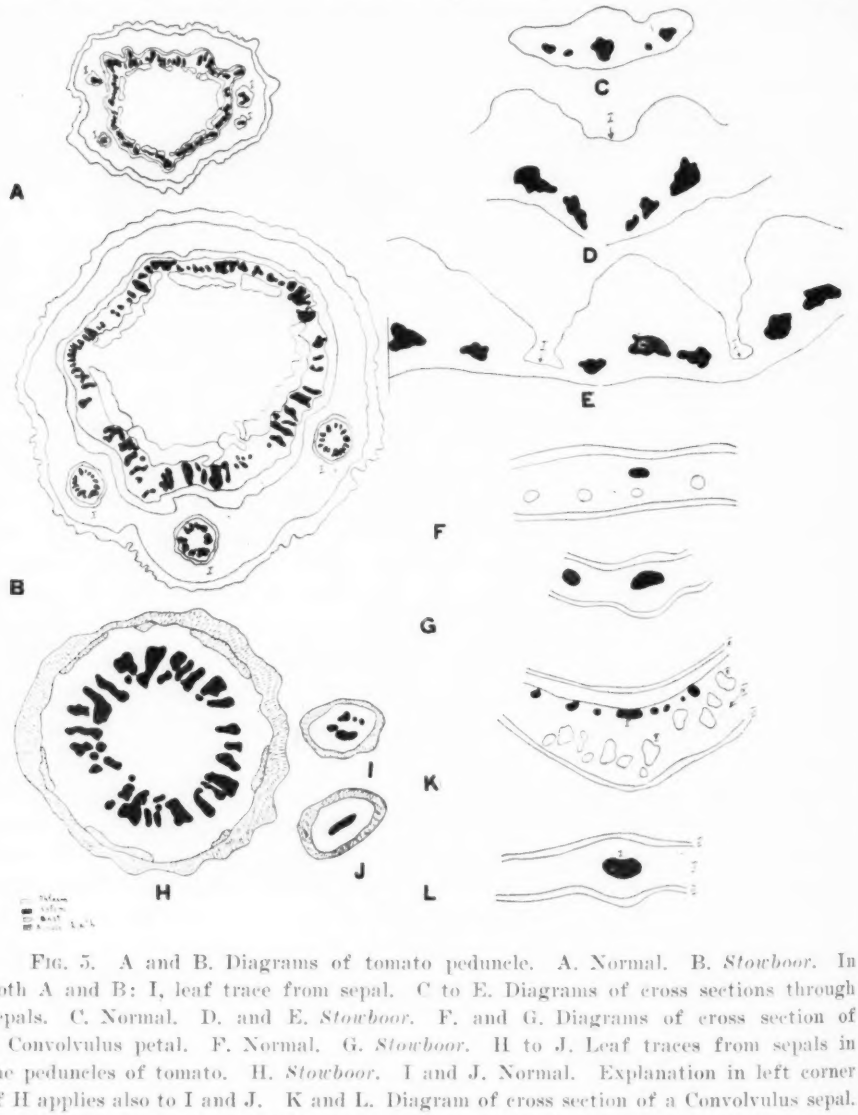

FIG. 5. A and B. Diagrams of tomato peduncle. A. Normal. B. *Stowboor*. In both A and B: I, leaf trace from sepal. C to E. Diagrams of cross sections through sepals. C. Normal. D. and E. *Stowboor*. F. and G. Diagrams of cross section of a Convolvulus petal. F. Normal. G. *Stowboor*. H to J. Leaf traces from sepals in the peduncles of tomato. H. *Stowboor*. I and J. Normal. Explanation in left corner of H applies also to I and J. K and L. Diagram of cross section of a Convolvulus sepal. K. Normal. L. *Stowboor*. In K and L: I, fibrovascular bundle; II, intercellular spaces; III, epidermis; IV, mesophyll.

of adaptable reaction. It is unwise to conclude that a seemingly adaptable structure has indeed an adaptable function.

The following fact deserves special attention. If we cut the peduncle of a diseased tomato plant 0.5 to 1 cm. below the flower we find a certain number of complementary steles near the epidermis, each stele being surrounded by a bundle sheath of its own. These steles are formed by the leaf trace of the sepals that stretch through the cortex of the peduncle. The number of such steles equals that of the fibrovascular bundles beginning in the sepals, and the cross section of the peduncle is structurally similar to that of the stalk of polystelic type (Fig. 5, B.) After the cross section had been treated with phlorglucin + concentrated HCl solution, the tissues of these complementary steles could be observed in greater detail. The center of the cross section (Fig. 5, H) was almost wholly filled with inner phloem.

The inner phloem was surrounded with xylem elements radially disposed. The xylem is followed by the exterior phloem, which, even in the peduncle, contains areas of bast. The layer quite at the periphery and separating the leaf-trace from the other tissues of the peduncle is an independent bundle sheath.

These findings forced us to consider carefully the structure of a normal peduncle in order to see whether there could be found there comparable structural phenomena. Indeed, the healthy peduncle was observed to contain traces of the same structure, but these were most insignificant. Only just beneath the sepal the cortex of the peduncle showed leaf-traces, each of the latter being surrounded by its own sheath. Very soon they joined the axial cylinder. These formations, occupying such a short stretch of space, were but slightly developed (Fig. 5, A and J).

The xylem occupied a small area, the inner phloem was hardly visible or even utterly lacking, and the outer phloem had no thick bast. It is known that polystely in dicotyledons occurs most rarely. It is interesting to note that, according to Solereder (8), polystely may be found in the pedicel of some Guttiferae, Terustroemiaceae, Malvaceae, Sterculiaeae, Meliaceae, and Moraceae.

THE FLOWER

We shall begin with the structure of the calyx. Figures 5, C, D, E and 2, E, F represent the corresponding areas of sepals of healthy and diseased flowers. Figure 2, E, F shows that the thickening of the sepals is not the result of an increased number of cells, but is caused by their greater volume. Passing now to the petals, we find the mesophyll is structurally very loose and undifferentiated in a healthy tomato plant. The petal of a flower attacked by woodiness almost completely lacks intercellular spaces or has very re-

stricted ones. The tissues are differentiated. The mesophyll cells are disposed lengthwise and remind one of the palisade parenchyma of the leaf. The rest of the tissue has cells of different shape that are closely packed together (Fig. 2, D). Such a development of the palisade tissue of the petal mesophyll may be regarded as the first stage of antholysis and transformation of the petals into leaves.[2]

The cross section through this axis of the flower above the sepal shows that the cells of the medulla, parenchyma, cortex, and collenchyma are crowded with grains of starch. The leaf traces of the petals have the same structure as the leaf traces of the sepals. For a certain time they form independent cylinders in the cortex. The difference is that of the duration and degree of development. The leaf traces of the petals are much shorter than those of the sepals. The leaf trace of a sepal is about 10.5 mm. long; that of the petal measures 4.5 mm. The diameter of the leaf traces of the sepals exceeds that of those of the petals.

THE POLLEN

The pollen of diseased plants deserves special attention. Having examined a great many pollen grains from the anthers of various flowers we did not find a single one of normal appearance. All of the pollen was sterile and without contents. The wall was shrivelled; often it was collapsed into a small lump. The normal pollen grain is of granular structure, spheroid, and possesses 3 spots through which pollen tubes grow. The structures of the anthers themselves show also appreciable differences. (Fig. 6, A and B).

THE FRUITS

The fruits suffer more than all the other parts of the plant. They are not suitable for food because of the strong lignification of the fibrovascular system; they become also quite tasteless.

Such fruits are easily distinguished from normal ones. They never turn quite red. Generally they are yellow with an orange or pinkish hue. In green fruit one may often find diaphanous spots formed by tissues filled with water, measuring 2×1.5 mm. or even more. The fruits are ribbed. In cross section a single fruit presents the following appearance: The placenta is quite free of pigmentation, being white. Only the peripheral parts of the pericarp are red, while the central parts remain as white as the placenta. The fibrovascular bundles are brown and so hardened that they can be plainly felt as one rubs the cut surface with the fingers. If one press the exposed cut ends, one experiences even a pricking sensation. A healthy tomato, when cut, is uniformly red; the fibrovascular bundles are white and just as soft as the cells of the placenta.

[2] It is known that woodiness of the tomato often lengthens the axis of the flower beyond the calyx. (Fig. 2, C and D.)

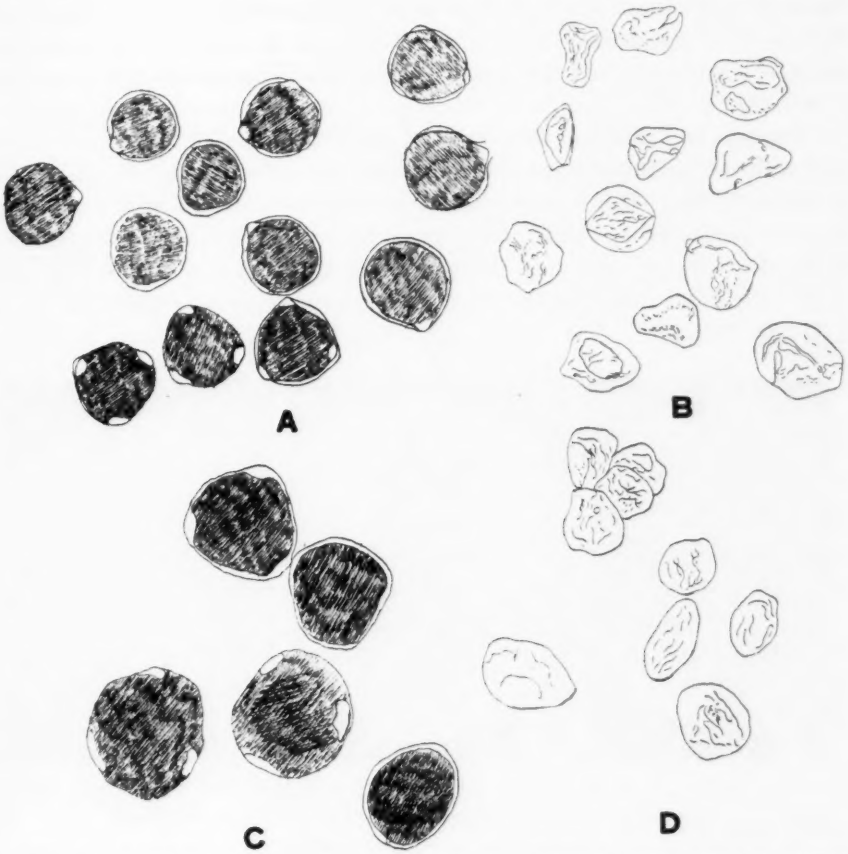

FIG. 6. A and B. Tomato pollen. C and D. Convolvulus pollen. A and C. Normal. B and D. *Stowboor*.

Microscopic examination of a fruit shows the starch content greatly changed in comparison with the above-mentioned parts of the plant. While diseased plants have little or no starch, the cells of healthy plants are filled with starch. While the fruit is yet quite young, there is no difference in its starch content, but when it has grown and measures 2 cm. or more in diameter we find that some of the cells of the diseased plant have very small starch grains and that these are present only in a greatly restricted number, while healthy fruits are rich in starch.

In examining a cross section through the fibrovascular bundles of a diseased tomato, it may easily be seen that lignification has affected the vessels only. There are 4 to 5 bundles in each fibrovascular tract (Fig. 1, A) in a normal fruit. A diseased fruit presents no such phenomenon. Besides the vessels, the lignification also extends to the other elements of the xylem,

disposed in the shape of a horseshoe or more or less in a circle. (Fig. 7, C). With a view to determining the constituents of the xylem in a more exact manner, the tissue of a healthy as well as of a diseased fruit was macerated in Schultze's solution.

The macerated tissue of a healthy fruit was mostly formed of vessels with spiral, thickened walls, of tracheids, and a few libriform cells (Fig. 4, D and 7, B). The tissue of diseased fruits consisted almost entirely of porous vessels that presented the appearance of shortened and greatly thickened cells. Spiral vessels in the macerated tissue of a diseased fruit were very scarce, but this tissue contained libriform cells in abundance. (Fig. 4, E and 7, A).

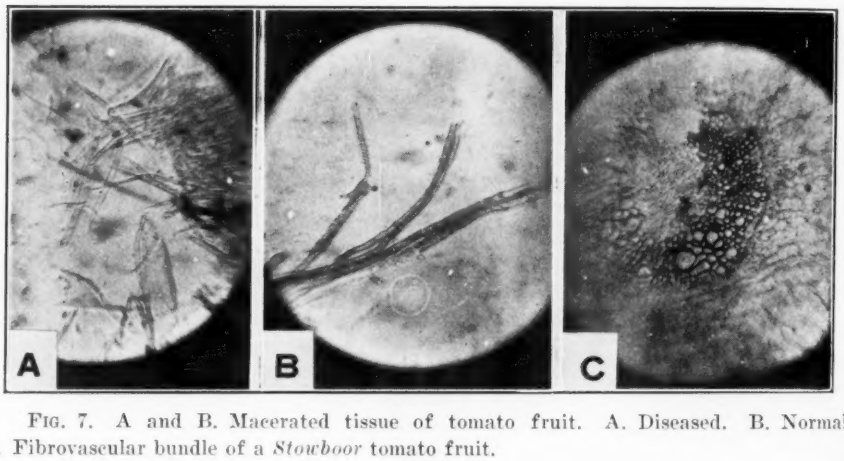

FIG. 7. A and B. Macerated tissue of tomato fruit. A. Diseased. B. Normal. C. Fibrovascular bundle of a *Stowboor* tomato fruit.

In the foregoing we have noted the pathological condition afforded by all the parts of a diseased plant. The separate parts of such a plant develop faster than those of a healthy individual. In the former we find completely developed fibrovascular bundles at an earlier stage of development than in a normal plant; the same may be said of secondary wood formation, and of the appearance of bast (in the stalk and pedicels). In the xylem we find almost no spiral vessels, proof of an earlier stage of development of the plant, whereas in a diseased plant all the spiral vessels become dotted or scalariform. In one instance a spiral vessel was observed even to turn into a porous one. Once we observed a vessel part of which had spiral thickenings while another part was porous (Fig. 4, C). These findings show that spiral vessels may change into porous ones.

The influence exerted by the virus on the inner phloem is especially evident; it is strongly developed in diseased plants and the phloem-cells are filled with a yellow substance, the nature of which, unfortunately, could

not be determined. This material may hinder the regular evacuation of organic substances, and, as a result, all of the parenchymatous cells of the stalk, the peduncle and the pedicel are crowded with grains of starch, while the fruits are quite void of starch or contain but little of it. It is interesting to compare these findings with the data recorded in the literature on pathological-anatomical changes that result from viroses. The filling of tissues with starch has been observed in different viroses. The monograph of V. Rischkov (3) summarizes the literature on this problem.

Here we need only to point out that in this instance the crowding of the tissues with starch was not accompanied by necrosis. This is definite proof of the fact that the conductivity of phloem may suffer even without any such gross changes. Probably the peculiar alteration of the contents of the sieve tubes, previously noted, is the outward expression of the loss of normal conductivity by the phloem.

Rochlina (5) noticed that potatoes affected with virus-diseases age promptly, developing sclerosis at an early date; but in none of the reported instances was this so strongly marked as in those reported by the writer. Of all the data recorded in the literature examined by the writer, the phenomenon as disclosed by the studies here presented, strongly recalls the changes in Passiflora caused by woodiness. In studies reported by others, hardening and tastelessness of the fruits are also characteristic of the disease, but in such cases woodiness is caused by excessive sclerosis of the peripheral layers of the pericarp parenchyma (2), while in the writer's case it is caused by peculiar alterations in the bundles.

The changes here described are unknown in other viroses and are specific for woodiness of tomato fruit. The symptom, therefore, possesses diagnostic value. Since the elements of xylem retain their characteristic structure, even in macerated fruits, the disease may be diagnosed even in mashed fruits or in those forming part of various dishes. The diagnosis is established on an anatomical basis. Unfortunately, Samuel, Bald, and Eardley (6) give only a short description of the anatomical alterations in tomatoes suffering from big bud. Still, their description proves that the morphological features of big bud and fruit hardening are just as identical to each other as are the anatomical symptoms. The Australian investigators have, like myself, observed hypertrophy of the inner phloem and crowding with starch grains in the tissues of diseased plants.

Although Samuel, Bald, and Eardley (6) do not mention any of the changes that I have observed in the xylem of the fruit, one may hardly doubt that they also occur in cases of big bud, for this disease is likewise characterized by a hardening of the fruit.

The study of anatomical symptoms of woodiness of tomato fruit and big bud has persuaded the writer that these diseases are identical.

In discussing the peculiar anomalies of histogenetic character observed in cases of woodiness we must, first of all, consider whether or not these changes in the fibrovascular bundles depend on a certain reduction in the size of the leaf blades of diseased plants. It is known that the kind of correlation existing between the leaf and the fibrovascular system of the stalk has been determined by experiment (10), as well as by observations (1). Zhakaja (10) noted the different changes that occur after the removal of elements of the node. Of these changes the increased development of the xylem is of special interest here. The changes of the leaf blade noted in woodiness of fruit are, however, comparatively unimportant and not comparable, for instance, to the filiform leaves seen in some mosaics of tomatoes, notwithstanding the fact that in the latter no profound alterations seemed to have taken place in the fibrovascular bundles. We, therefore, are bound rather to explain the anomalies of the xylem and the phloem by a specific action of the virus than by changes in the node elements.

PATHOLOGICAL-ANATOMICAL CHANGES IN WOODINESS OF FRUIT IN BINDWEED, CONVOLVULUS ARVENSIS

On tomato plantations and in their neighborhood there grows a weed named *Convolvulus arvensis* or bindweed. Very often it is attacked by a disease closely resembling woodiness of fruit (*Stowboor*). The diseased plants bear small, round leaves. Just as in the case of diseased tomato plants, their leaves are chlorotic, especially at their margins, which often curl upwards. A diseased bindweed seldom blooms and, when it does, its flowers show changes typical for stowboor. I have studied the anatomy of such plants.

THE ANATOMICAL STRUCTURE OF A BINDWEED FLOWER

In healthy individuals the superficial epidermis of the petals consists of one layer of cells, slightly drawn out in the direction of the breadth of the petal. The mesophyll has 9 to 10 layers with rounded, small, compactly disposed cells. Between the mesophyll and the epidermis there is a row of rounded cavities that probably extend along the whole length of the petal. The lower epidermis consists of a layer of cone-like cells with their apices directed outward. The fibrovascular bundles contain 2 to 3 vessels each and their phloem is hardly discernible (Fig. 5, F and G).

Cross sections of petals of a bindweed suffering from stowboor remind one of those of healthy petals. They are rounded and crescent-shape. Their mesophyll consists of roundish cells, lying close together. There are no such lacunae as we have seen in the petals of healthy plants. The size of the fibrovascular bundles in the center of the mesophyll is greater than in normal individuals, and the mesophyll cells are packed with chloroplasts.

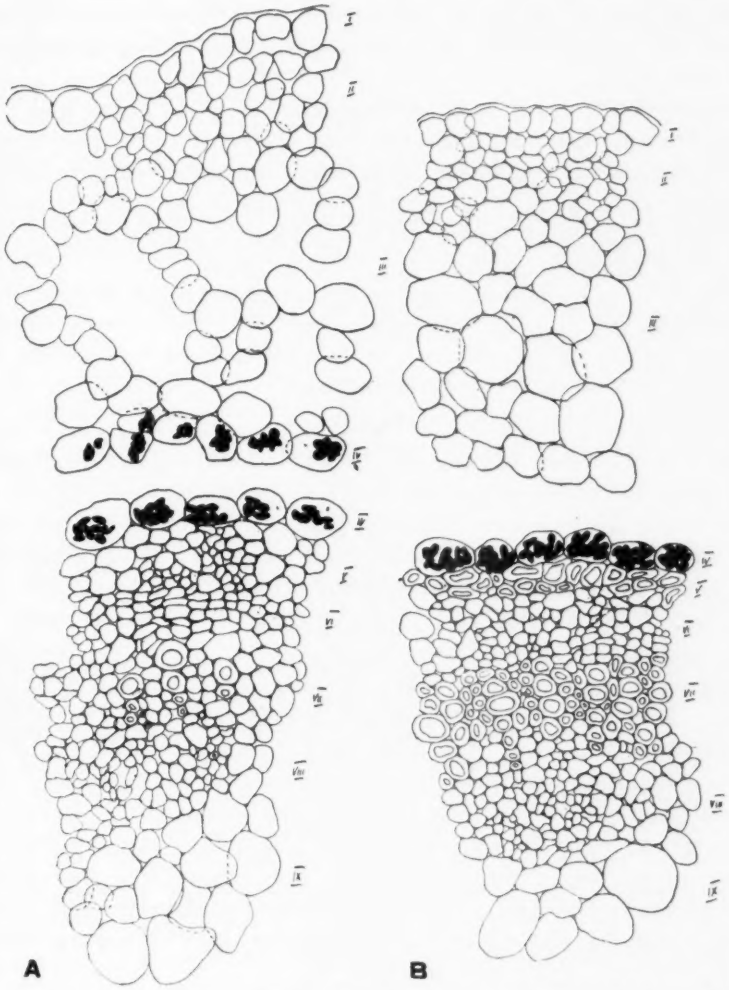

FIG. 8. A. and B. Cross section through peduncle of Convolvulus. A. normal. B. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, cortical parenchyma; IV, bundle sheath; VII, xylem; VIII, inner phloem; IX, pith. In A: V, outer phloem; VI, cambium. In B: V, bast; VI, outer phloem.

The sepals of a healthy Convolvulus are crescent-shape in cross section. In the middle of the crescent the mesophyll consists of 13 to 14 layers of cells, while at the ends there are but 1 to 2 layers. The cells are rounded and lie close together. Nearer to the epidermis are lacunae of greater size than those of the petals. There are about 35 of them in a single section and they all lie in series. Next to the mesophyll there follow 3 to 4 cell layers whose walls are very thick; then comes an epidermis of rounded cells

forming a layer. The fibrovascular bundles are situated very close to these thick-wall cells. The bundles are poorly developed, having no more than 8 vessels each.

The cells of the upper and lower epidermises of the sepals of bindweed suffering from stowboor are quadrangular. Their outer walls are cutinized. The mesophyll consists of 6 compact layers of quadrangular cells filled with chloroplasts and starch. The midrib has 20 to 25 vessels surrounded by mechanical, nonlignified tissue and the phloem is poorly developed. The region of the midrib is swollen.

The midrib has 5 fibrovascular bundles, one of which is in the center with two smaller ones on each side (Fig. 5, K and L).

THE PEDUNCLE

The structure of the peduncle deserves special attention in a study of the bindweed, whether healthy or diseased. A cross section through a healthy peduncle shows an epidermis of a single layer of cells; 2 to 3 layers of assimilating tissue; a loosely formed parenchyma with large intercellular spaces; and 9 to 10 fibrovascular bundles forming a ring. The existence of a great many intercellular passages in the peduncle of Convolvulus is most interesting. They develop as they do in the stalk of water weeds. This likewise is characteristic of other kinds of Convolvulus, as, for instance, *C. cantabricus*, and of such other members of the Convolvulaceae, as Ipomoea. I have not been able to find in the literature the description of a like peduncular structure. Van Tieghem (9) does not mention it, although he gives a detailed description of the structure of the peduncle. In the systematic anatomy of Solereder (8) there also is no mention of it. Under the influence of virus the structure of the peduncle of a plant attacked by *stowboor* undergoes profound changes. The intercellular spaces of the cortical parenchyma utterly disappear. Thick bast develops and the xylem forms a continuous ring with numerous lignified cells of mechanical tissue. (Fig. 8).

The writer presents here the original record of the investigation.

Normal Plant

(1) Single layer of epidermal cells filled with protoplasm; 0.1395 mm. in diameter. Assimilatory tissue, consisting of 2 layers of cells, 0.1396 to 0.155 mm. thick, with chloroplasts and starch. The cortical parenchyma has enormous air-filled cavities; this parenchyma has a thickness of 1.1345 mm. to 0.93 mm.; inside the cells there is protoplasm and a few starch grains, visible after treatment with iodine. The cells of the one-layer bundle sheath are crowded with starch grains; it measures 0.093 to 0.124 mm. In the middle of

Stowboor Plant

(1) Single layer of empty epidermal cells of 0.124 to 0.099 mm. in diameter. Assimilatory tissue, 2 to 3 layers, 0.124 to 0.155 mm., with chloroplasts. The cortical parenchyma is 0.5425 to 0.52 mm. broad; its cells are smaller than in the healthy plant and quite without any intercellular spaces. One layer of thick bast, measuring 0.0775 mm. The outer phloem is 0.217 to 0.2325 mm. broad. The xylem is continuous with a radius of 0.31 to 0.4135 mm. The inner phloem is 0.275 to 0.341 mm., large, continuous; sieve tubes plainly visi-

the peduncle (2.728 to 2.371 mm.) are large cordate cells with wide intercellular spaces; the cells contain protoplasm and several grains of starch. The core is surrounded by inner and outer phloem and xylem. There are 1 to 2 areas of xylem with 3 to 4 vessels each. Most of the latter are spiral-shape; also, there are 5 areas with 10 to 13 spiral vessels. No more than 3 vessels are met with in a xylem tract.

ble. The core measures 0.93 to 1.24 mm. The cells are big, without contents; intercellular spaces small. No starch.

The investigation of bindweed affected with *stowboor* proves that (1) Under the influence of the virus the peduncle becomes much hypertrophied. (2) The xylem develops more intensely than in a healthy Convolvulus plant and forms a continuous ring in the peduncle. (3) The aboveground parts of the plants contained more starch than was present in healthy ones. (4) The leaf mesophyll is without intercellular spaces. (5) The pollen of a Convolvulus plant affected with *stowboor* is sterile. (Fig. 6, C and D). The above-mentioned symptoms are characteristic also for woodiness of tomatoes. Naturally, only artificial infection of tomato plants with *stowboor* from a diseased bindweed would definitely prove the identity of both diseases. In conclusion, I am much indebted to Prof. Dr. V. L. Rischkow for his very helpful suggestions and advice.

SUMMARY

The writer has studied the anatomical structure of all the parts of tomatoes affected with woodiness of fruit, as well as the flowers and peduncles of a Convolvulus suffering from a disease of the same type.

In the tomato the following facts were observed:

The stalks of a tomato plant affected with woodiness of fruit develop faster than those of a healthy plant. The interfascicular wood and the ring of bast appear earlier than in the healthy plant.

All the tissues of the aboveground parts undergo a more or less pronounced hypertrophy.

Hypertrophy is especially well expressed in the inner phloem, which occupies, in cross section, an area equal to that of the outer phloem and xylem taken together.

The cells of the inner phloem are filled with a pale yellow substance.

The vegetative parts of a tomato are filled with starch grains; the leaf blade forms an exception to this rule. In the fruits, starch disappears very early, contrary to the condition observed in healthy plants.

In cases of woodiness of fruit, the structure of the mesophyll of the leaves and petals shows almost no intercellular passages in the mesophyll of diseased tomatoes.

The thickness of the peduncle and the pedicel of diseased tomato plants, as well as the degree of development of the mechanical tissues in the same, exceed those of a healthy plant, although the fruits are smaller.

In the upper parts of the peduncle and pedicel and in the anomalously lengthened axis of the flower, we observe a polystelic structure.

The pollen of the flowers of plants suffering from woodiness is sterile.

In diseased fruits the fibrovascular bundles are strongly lignified.

The wood of these bundles contains much libriform tissue, which is lacking in xylem of healthy fruits. In diseased fruits, spiral vessels are replaced by porous vessels.

The changes in a diseased bindweed are the same as in a *stowboor* diseased tomato: reduced intercellular spaces, intense development of xylem, numerous starch grains in the tissue, etc.

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THE COTTON ROOT ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM, PARASITIC ON THE WATERMELON, CITRULLUS VULGARIS

KARL D. BUTLER[1]

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INTRODUCTION

Phymatotrichum omnivorum (Shear) Duggar, the destructive fungus that causes Texas root rot (cotton root rot), probably is the most important plant parasite in Arizona and the semiarid Southwest. The fungus has a known host range of more than 550 wild and cultivated species (22). The watermelon, *Citrullus vulgaris* Schrad., has been listed by Taubenhaus and Killough (25) as completely resistant to the fungus. Later Taubenhaus, Dana, and Wolff (21) list as immune or resistant species *C. vulgaris* and all other members of the Cucurbitaceae except the summer squash, *Cucurbita pepo* L., variety *condensa* (Bailey).

Repeatedly, infection of the watermelon with *Phymatotrichum omnivorum* has come to the notice of the Department of Plant Pathology of the University of Arizona. On June 29, 1930, S. L. Owens, County Agent of Yuma County, submitted specimens of dying vines from a field in which watermelons had followed alfalfa and were wilting in the spots where Texas root rot had killed alfalfa the previous year. From the dying vines *P. omnivorum* was obtained. The affected variety was not recorded. During the summer of 1932 two cases were reported. The first case came on July 7 from C. J. McCash, Assistant County Agent of Pima County. Later, M. M. Evans found watermelon plants affected with the disease. In both cases the variety of melon was Iowa King. Recently a 5-acre field of Klondike watermelons near Tucson was found to be badly spotted with Texas root rot. The infected area, totalling about two acres, was a total loss to the grower. A number of young peach and pecan trees planted in this field also died of root rot. Other cases have been reported from the Salt River Valley and the Casa Grande district. The reports stimulated an investigation to ascertain the degree to which watermelons are susceptible or resistant to Texas root rot; also an investigation of the penetration of the parasite and the effect of mixed cultures.

[1] The writer gratefully acknowledges his appreciation of the assistance given him by the following members of the Department of Plant Pathology of the University of Arizona: Dr. R. B. Streets, who supervised the investigations, Mr. M. M. Evans, and Dr. J. G. Brown.

RESISTANCE

General. Although the breeding and selection of plants resistant to or immune from disease is the ideal method of plant-disease control, little is known about the absolute cause or basis of true resistance in plants. Different plants vary greatly in their resistance to disease. One or more factors may enter into a given case of resistance. The factors may be exceedingly complex, involving a detailed study of morphological systems and physiological processes. Resistance in one case may be due to the presence of a substance in the host that is toxic to the parasite; in another case it may be caused by an entirely different factor. Obviously, practically every problem of resistance in plants is necessarily a specific one and must be attacked as such.

Resistance of Plants to Phymatotrichum omnivorum. Although Texas root rot has been investigated since 1888 (16), comparatively few resistant plants have been found. Since the fungus is indigenous to the Southwest (10) and its sclerotia, which may remain viable for several years, are present in infested soils (15), the problem of resistance obviously is important. Recently it has been reported (13) that strands of the organism remain viable and infectious in certain soils for 3 or more years. This discovery adds to the complexity of the problem.

The literature presents a limited amount of information on the nature of the resistance of plants to Texas root rot. However, many workers have studied in detail various phases of the disease and their contributions are valuable. As early as 1888 Pammel (16) advocated the rotation of crops on diseased soil. In his original paper on root rot he said: "Corn, sorghum, millet, wheat, oats, and other members of the grass family do not die from this cause, and consequently should be used after cotton." In 1907 Shear (18) (19), with field experiments, showed the value of proper crop rotations. With a 2-year rotation of corn and wheat, he obtained a decrease of 95 per cent of the cotton dying from root rot to 5 per cent after the rotation. Similar recommendations are given by McNamara (12).

There has been some difference of opinion as to the degree of resistance or immunity of various monocotyledonous plants. King and Loomis (11) reported strands of *Phymatotrichum omnivorum* on roots of the date palm and on grass roots, but did not prove that the fungus gets nourishment from such roots. On the other hand, Taubenhaus and Ezekiel (23) (24) showed rather conclusively that monocotyledons are very resistant to the fungus. They reported that, although date palms, corn, sorghum, Johnson grass, Bermuda grass, and other grasses were interplanted with cotton and all plants were inoculated, only the cotton plants died. Depressed, deep brown lesions were found on roots of some monocotyledonous plants, but isolations from these failed to yield *P. omnivorum*.

Studies on the physiological basis of resistance have been published by Ezekiel, Taubenhaus, and Fudge (7). They show that cultures of *Phymatotrichum omnivorum* grow much more luxuriently on cotton media than on corn media. The heaviest growth of the fungus was found when the largest supply of dextrose was available. In a later report by the same authors (8) very slight fungus growth was obtained on undiluted juices of monocotyledons (corn, onions, cannas, and nut grass), although heavy growth was evident on diluted juices of susceptible dicotyledons (cotton, carrots, turnips, and sweet potatoes). Furthermore, upon dilution of the juices from monocotyledons the growth of the parasite was not inhibited. The experiments are interpreted to indicate that monocotyledonous plants contain a substance or substances that, in sufficiently high concentrations, can inhibit growth of the root-rot fungus, and that the resistance of the plants is based, at least in part, upon the presence of the substance or substances.

More recently, Moore (14) has shown that corn, wheat, and barley media do inhibit growth of *Phymatotrichum omnivorum*. She states that the inhibition is due to the presence of labile, water-soluble substances in the corn roots, and that these substances in small quantities may act as stimulants.

It is interesting to note that the Turk's-cap hibiscus, *Malvaviscus conzattii* Greenman, is the only plant out of 82 species belonging to the family *Malvaceae* that has shown no natural infection with the root-rot fungus under field conditions. Some 1-year-old plants became infected following inoculation, but they recovered. The factors involved and the nature of this resistance have not yet been revealed (1). Moore (14) believes that in this case resistance may be due to the presence of labile substances similar to those present in monocotyledonous plants.

Certain other plants, such as the hackberry, live oak, pomegranate, and many others (21), are known to be highly resistant to root rot, but the nature of the resistance is still obscure. For several years, in Texas (22, 6) as well as in Arizona, a large number of varieties of cotton and selections of individual strains have been tested for possible resistance to root rot. Many of these plants have been eliminated because of susceptibility, but the remaining ones are still under test. Nevertheless, no similar work has been published in regard to the watermelon plant.

EXPERIMENTAL WORK

Materials. The watermelon hosts used in the studies here reported consisted of the wilt-resistant Iowa King, which is becoming a popular variety in Arizona; the wilt-resistant varieties, Iowa Belle and Pride of Muscatine; and the wilt-susceptible Black-seeded Klondike. With the exception of

Black-seeded Klondike, the plants were grown from seed originally obtained from the Iowa Melon Growers' Association. Acala cotton seedlings, grown from seed supplied by R. B. Streets, of the University of Arizona, also were studied to a limited extent because of their known susceptibility to the Texas root-rot fungus.

As a source of inoculum, sclerotia from Goodyear and Sacaton, Arizona, were obtained from C. J. King, of the United States Field Station at Sacaton. Several sclerotia were surface-sterilized 3 minutes with a 1–1,000 mercuric chloride solution placed on sterile filter paper and transferred to potato-dextrose-agar slants. No growth was evident until the third day, and then it was very slow and scant. It was not uncommon to find a mixed culture growing from a single, surface-sterilized sclerotium. The pure culture from a single sclerotium was increased on steamed carrots, plugs of watermelon rind, sweet potatoes, potato-2 per cent dextrose agar, and other media.

Methods and Results. The field experiments were conducted at the New University Farm near Tucson, at an elevation of 2300 feet. The soil is a light sandy loam with a moisture equivalent of 12 to 15; the alkalinity is variable, although the differences between the first and second feet in any one case were not great; the average pH was found to be 8.48 with a minimum and maximum of 8.3 and 8.65, respectively. No temperature records are available for the plot studied, but air and soil temperatures recorded on the campus are given below (Table 1). Over a period of several years the air temperatures prevailing on the campus are known to average 4 or 5 degrees F. higher than those prevailing at the Farm.

TABLE 1.—*Air and soil temperatures, University of Arizona campus, Tucson, during period of field inoculations*

	Air temperatures (U. of A. Weather Bureau)							
	1932				1933			
	Aug.	Sept.	Oct.	Nov.	May	June	July	Aug.
Max.	105	103	93	84	103	111	106	
Min.	64	57	36	32	38	52	67	
Mean	85.2	80.6	67.8	60	67.5	80.8	87.7	
	Soil temperatures: Mean temperatures at depth of 4 inches based on weekly averages (recorded by M. M. Evans)							
Max.	82	83	67.2	61	69	84	83.2	
Min.	76	74	62	54.5	62	72	77	
Mean	79	78.6	64.6	57.75	65.5	78	80.1	

Cotton had been grown on the plot for several years and active root-rot spots were present. Considerable damage occurs annually in adjoining

fields of alfalfa and cotton. No sclerotia of *Phymatotrichum omnivorum* have been found in the field in which the plot was located.

On September 30, 1932, 40 days from date of planting, 39 watermelon plants were inoculated by placing infected cotton roots in the soil in the center of each hill. Eighteen plants were Iowa Belle and 21 were Pride of Muscatine. Two large plants of the Muscatine variety were carefully excavated on October 22 and the roots were thoroughly examined, but no characteristic strands or lesions were found. Six days later the roots of 2 Iowa Belle plants were examined with similar result. On November 5 one large plant of each variety was examined and found healthy. Fourteen days later (50 days after inoculation) the plants remaining in the plot were removed

FIG. 1. Watermelon vine killed by *Phymatotrichum omnivorum* after inoculation with a pure culture of the fungus.

3 days after a killing frost. Precautions were taken to obtain as complete root systems as possible. Examination in the laboratory disclosed typical lesions with connecting strands on roots of both varieties of watermelons. Portions of infected roots were prepared for cytological study and other pieces were cultured. Attempts to isolate the fungus from this material were not successful.

On the same soil watermelon seeds of Iowa Belle, Iowa King, and Black-seeded Klondike were planted in hills about 2 feet in diameter on April 28, 1933. All hills except 2 of each variety were inoculated on June 19. The inoculum was a pure culture of *Phymatotrichum omnivorum* grown on steamed carrots in 750 cc. flasks for 39 days. The inoculum was removed from a flask and placed in a hole approximately 6 inches deep in the center of each hill. At this time alfalfa plants in the adjoining field were wilting from root rot. Up to and including July 7, 18 days after inoculation, there was no evidence above ground that disease was present. On this date several plants were carefully removed and examined. Strands and small lesions were found on the roots of 3 plants. Pieces of roots with lesions were cultured in the laboratory and *P. omnivorum* was isolated from 2 cultures.

On October 26 (about the end of the growing season) all of the plants in this experiment were dead or dying from root rot except one of the check plants. The other check plants had by this time contracted the disease from adjacent inoculated plants. Of a total of 36 plants, 35 became infected with root rot.

In the previously described experiments the watermelon plants were artificially inoculated; the disease also occurred naturally in the field. On the watermelon, like most other hosts, the disease results in a sudden wilt-

PLATE I

A. a. Iowa King pure culture seedling 25 days old. Dead from attack of *Phymatotrichum omnivorum*. Note the brown roots with fungus. ×1.5. b. Check; white clean roots against tube. B. Lateral root from Black-seeded Klondike in field. Note lesion and strands. ×1.5. C. Iowa King seedlings on agar slants. In a and b Phymatotrichum has surrounded roots of seedlings and is growing on them; Ck shows vigorous sterile conditions of check. ×1. D. a. Pure culture of Phymatotrichum on 2 per cent potato-dextrose agar. b. Inhibiting effect of *Trichoderma lignorum* on *P. omnivorum*. 7 days from date inoculated. a and b inoculated at same time. ×.5. E. The root system of a diseased watermelon plant of the variety Klondike. ×.15. F–H. Photomicrographs of watermelon roots infected with *P. omnivorum*. F. Shows that the fungus filaments are largely intercellular. From root on agar culture. 5 days. Cortical tissue. ×400. G. Wedging of fungus on outside of large root from field, Black-seeded Klondike variety. Section 2 inches below soil line. ×25. H. Cross section of root 48 hours after inoculation. Iowa King variety. ×10. Photographs A and C by R. B. Streets; all others by M. M. Evans.

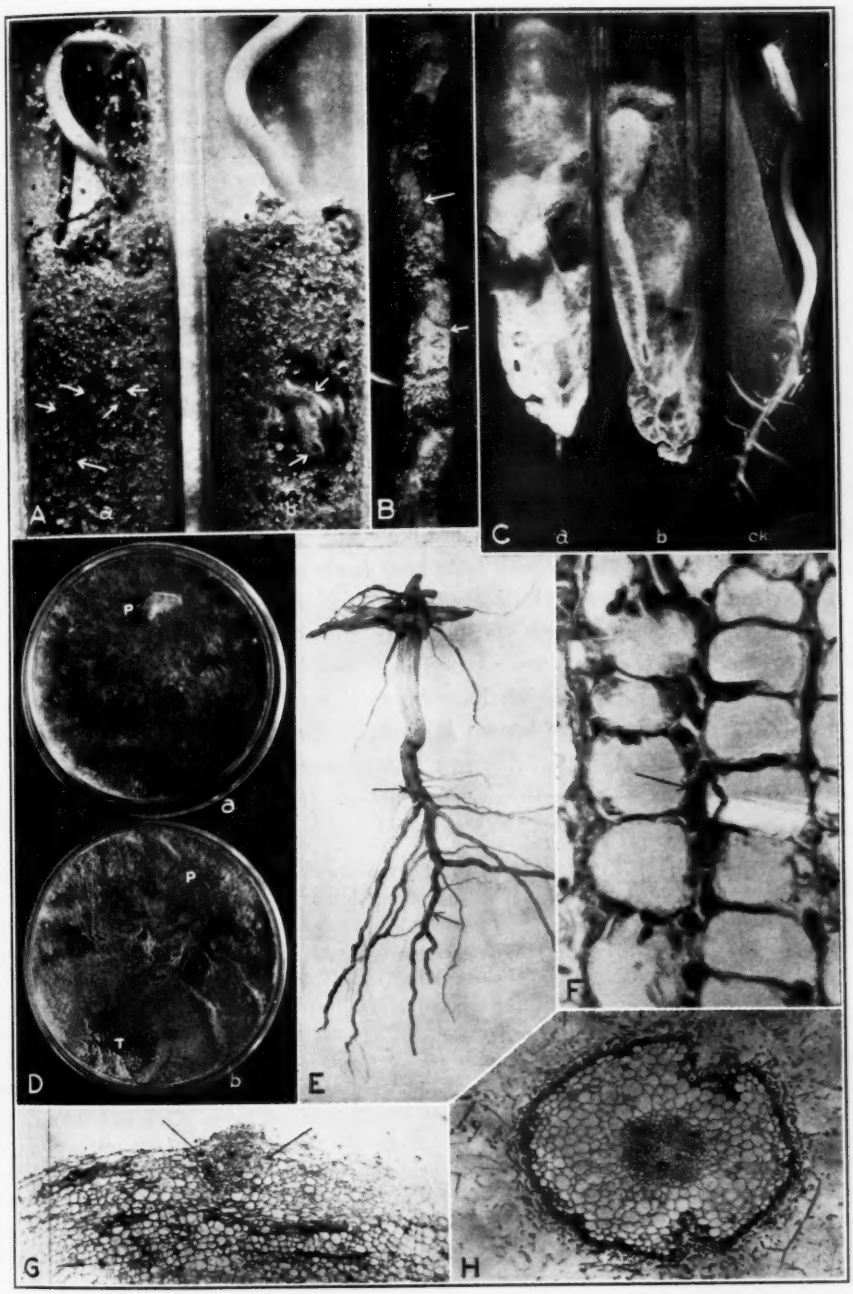

PLATE I

ing and drooping of the leaves. On July 11 one plant of the Klondike variety wilted (Fig. 1). The affected plant in this case was somewhat pale yellow. The root system was removed from the soil to the laboratory, together with that of an unwilted plant from the same hill.

When examined the root system of the wilted plant showed late stages of infection with the Texas root rot fungus (Plate I, E). The taproot was dirty brown and the rotted condition was more advanced toward the tip, indicating that early infection probably had taken place at least 12 to 14 inches below the surface of the soil. Typical, brownish strands of the fungus were prevalent on the surface of the taproot and lateral roots. On the first 2 inches of the taproot there was no external evidence of infection, but at 3 inches there were slight signs. Some parts of the root system were so completely rotted that the soft, slimy cortex could be easily removed from the woody central cylinder.

Very few lateral roots were present on this specimen, although the rotted remains of many were found in the soil. There were more lateral roots on diseased plants of Black-seeded Klondike (Plate I, B). Infection was generally more severe on the lateral roots near their attachment to the main root than farther out. This may indicate that infection had spread from the main root to the laterals, although the opposite condition was sometimes found. Different parts of the roots were killed in Gilson's reagent for further study.

The excavated plant, which bore no visible symptoms of disease above ground, was found to have brown strands of the Phymatotrichum fungus on its roots; a few small, light brown lesions were present.

PLATE II

A. A single hypha growing between two epidermal cells. Root of Iowa King in contact with inoculum 48 hours. B. Penetrating hypha between two cells then into a cell and in contact with the host cell nucleus. Note constriction of hypha at point of entry. C. A later stage showing the development of the hypha in the host cell in direct association with the cytoplasm and the exceedingly small host nucleus (*n*). Also note the cross section of hypha growing intercellularly. D. Hyphae wedging and pushing into root. Note multinucleate fungus cells; 4 nuclei in one case. E. The host cell wall has been stretched and, although the fungus is exerting considerable pressure, penetration has not yet been accomplished. F. Penetration has been accomplished just as the hypha had begun development intercellularly. G. Direct penetration has taken place and the hypha has branched into an haustorium-like body. Note the accumulation of mycelium on outside of host cell; also the direct contact of host cell cytoplasm and fungous hyphae. H. Cortical parenchyma cell isolated by enveloping hyphae, 5 days from time inoculated. Note the shrinking of the protoplasm from the cell wall. I. Coiling of *Trichoderma lignorum* filament (*t*), around *Phymatotrichum omnivorum* filament (*p*). Note vacuolate condition of the root-rot-fungus. J. *Trichoderma lignorum* filament inside of root-rot aerial hypha. The Phymatotrichum filament is without protoplasm when in association with Trichoderma. All × 853.

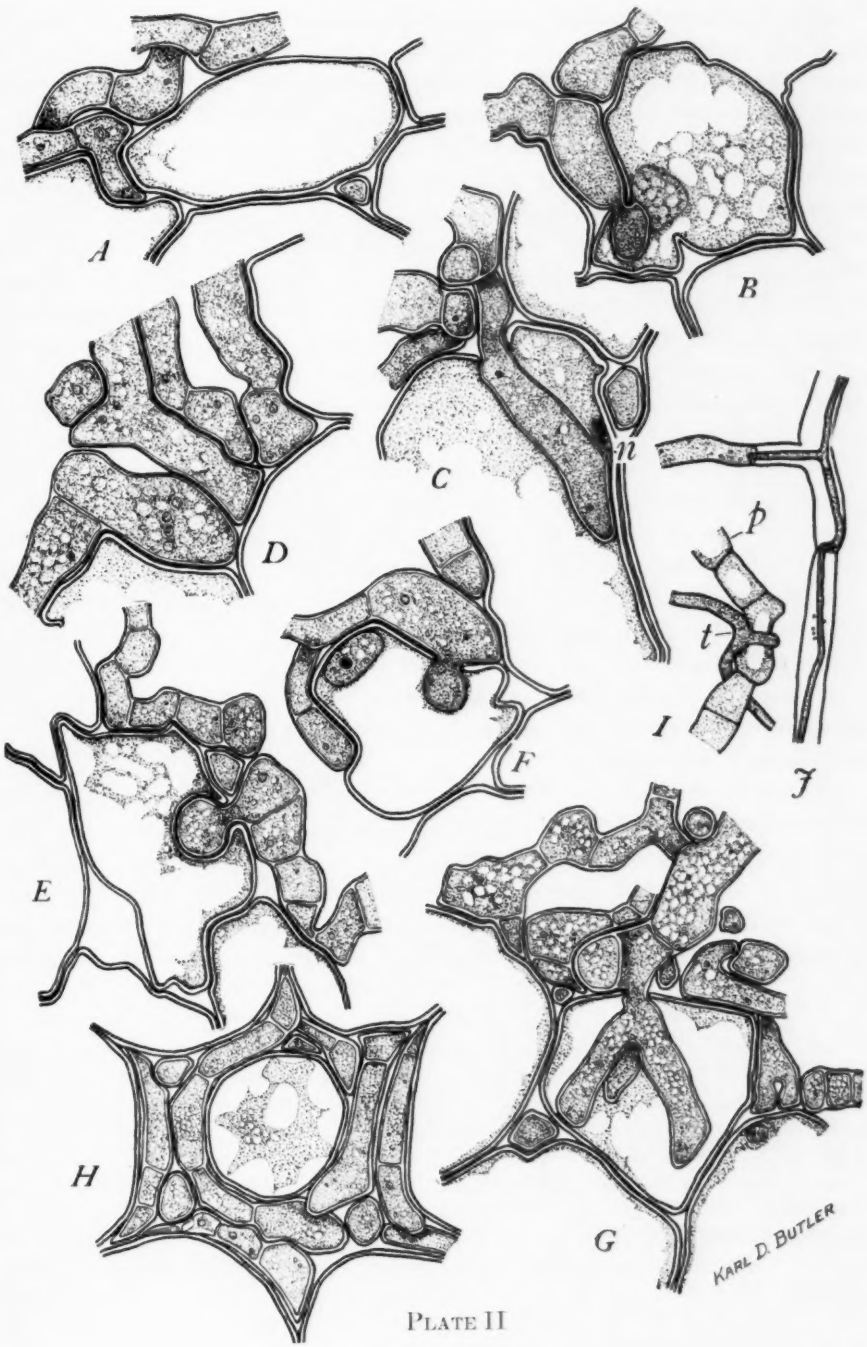

PLATE II

On the stained sections made from this material 2 inches below the soil line, the wedging, mass action of the penetrating fungus was evident at many places (Plate II). The parasite had advanced through and between only 6 to 8 rows of cortical cells, but the mycelium was rather uniformly distributed in the infected area. Fungus filaments were prevalent in the vessels in the more severely attacked parts of the roots, and the cortical tissue was collapsed and dead.

In general, it appears that the cortical cells nearest the invaded cells contain less protoplasm than do those farther removed; also, cells penetrated with hyphae often show plasmolysis (Plate III, F). In some cases this had begun where a hypha was in contact only with the outside of the cell. A marked difference was noted between the nuclei of invaded and noninvaded cells. Those in invaded cells were distinctly smaller, stained much more deeply, and appeared to be in a state of inactivity (Plate III, C and F). The nuclei in noninvaded cells not only were larger but also clearly showed the usual fine nucleoplasmic network and contained a prominent nucleolus (Plate III, E). Measurements of greatest nuclear diameters gave the following results: in the noninvaded cells the maximum diameter was 14 μ, the minimum, 8 μ, and the average 11.8 μ; in invaded cells these diameters were 6.5 μ, 3 μ, and 4.8 μ, respectively. Only nuclei in noninvaded cells 3 or less cells distant from the invaded ones were measured. The nuclei of invaded cells averaged less than half the diameter of those of noninvaded cells. The manner of fungal invasion of the cells was investigated in the laboratory.

Penetration Studies. Detailed cytological studies on penetration by various fungi have been discussed (20, 2, 4). There appears, however, to be no report at hand of any similar study of the Texas root-rot fungus. The early penetration stages of the fungus having never been figured or described, they are presented here. The most complete description of the penetration of the parasite is that given by Peltier, King, and Samson (17).

PLATE III

A. Fungus hyphae passing from one cell to another in cortex. Invasion from both intra- (*a*) and intercellular (*b*) spaces. Note scant protoplasm in host cell. Two hyphae entering through same hole in cell wall. B. Branched filaments in a lateral root growing between parenchymatous cells toward the main root. 5 days from time inoculated. C. An invaded cortical cell in a mature watermelon root from the field. Smaller and denser nucleus than in an uninvaded cell (E). Filament in direct contact with vacuolate host protoplasm. Also a fungus nucleus appears to be in the process of division. D. A branched filament in xylem cells of a 25-day-old plant. E. A noninvaded cortical host cell only 3 cells from invaded tissue. Note the large nucleus with the fine nucleoplasmic network. F. A cortical cell permeated with fungus filaments. Note the extremely small host nucleus, the shrinking of protoplasm from the cell wall and the scant protoplasm. B, E, F are from field material. × 853.

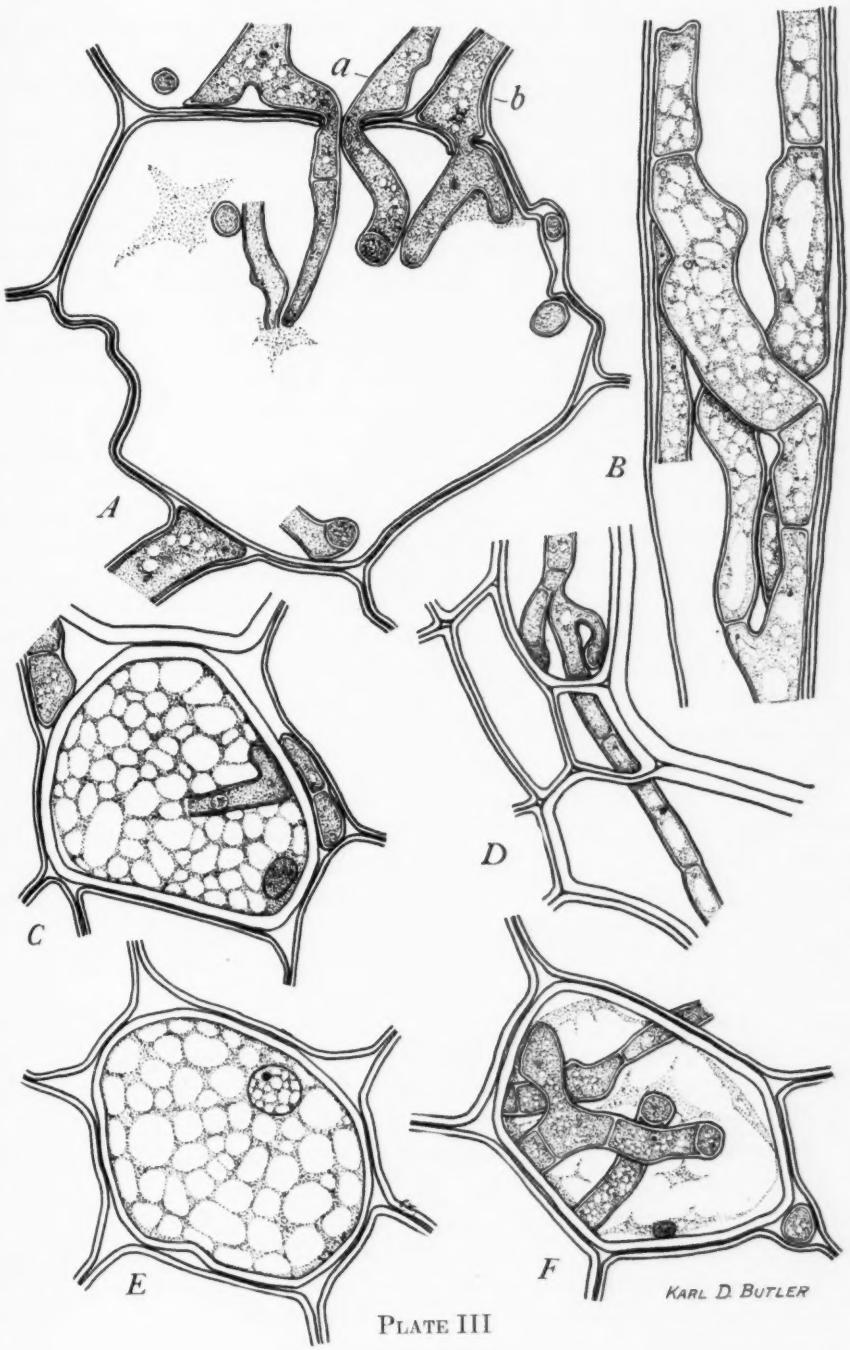

PLATE III

In their work, however, they did not use pure cultures and the field was covered only in a preliminary way.

Penetration of the fungus into the watermelon plant was studied with seedlings grown on sterile filter paper and potato-2 per cent dextrose-agar. Seeds of Iowa King were surface-sterilized by a 3-minute immersion in 1:1,000 mercuric chloride solution, rinsed thoroughly in sterile distilled water, and arranged on wet filter paper in sterile Petri dishes so that all roots could grow in the same direction. The dishes were now placed in a vertical position in an incubator at a constant temperature of 28° C. At the end of 48 hours the seeds that had germinated were transferred with flamed forceps to potato-2 per cent dextrose-agar plates, inoculated with *Phymatotrichum omnivorum* 4 days earlier. The inoculum was a 15-day pure culture on steamed carrots. The plants were placed vertically with the roots in the same direction. The roots varied from 5 to 10 mm. in length.

The cultures, divided into 5 groups, were observed under the binocular microscope at intervals of 8 hours. Group 1 was removed from the incubator after 24 hours; groups 2, 3, 4, and 5 at the end of each succeeding 24-hour period, respectively. Upon removal the roots of the seedlings were placed in Gilson's fluid and maintained 12 hours at 70° C. The material was then washed, dehydrated, cleared, embedded in paraffin, sectioned 5 to 10 μ thick, and stained. Heidenhain's iron-alum haematoxylin proved very satisfactory, especially when sections were counterstained with safranin.

Examination of the seedlings with the binocular microscope showed that the large-cell type, and more often the young strand types of hyphae (so designated by Duggar (3)), had started to grow around and on the roots after 8 to 16 hours. After 24 hours the entire root often was surrounded by the fungus, especially near the root-tip. No actual penetrations were observed with sections of 24-hour cultures. Nevertheless, some wedging action had begun.

The following 3 modes of fungal invasion of the young seedlings were noted: (a) by direct penetration into a cell, (b) by growing between two epidermal cells and finally into a cell, and (c) by the wedging of several or many hyphae, thus causing a lesion.

The roots of seedlings that had been in contact with the culture of the fungus for 48 hours were completely surrounded by mycelium (Plate I, H). The cells of the hyphae were rich in protoplasm, usually large, of irregular shape, with rather thick walls, and multinucleate. It was not uncommon to find 3, 4, or more nuclei between septa (Plate II, D).

Actual penetration had taken place in many instances after 48 hours, (Plate I, H). Although direct entrance into an outer cell was not unusual,

it was less common than intercellular penetration (Plate I, G, and Plate II, A, D). In either case it was evident that pressure was necessary to initiate final penetration. Often a hypha progressed between the cells and finally entered. Apparently, a softening action took place at the point of entry in addition to the mechanical pressure (Plate II, E). In no case was there evidence of any tearing or irregular rupture of the penetrated wall. The portal of entry was smooth and nearly always smaller in diameter than the invading hypha; consequently, that part of the hypha that passed through the host cell wall was usually smaller than at either side of the wall (Plate II, B, and Plate III, A). There were many separate and distinct invasions.

The fungus invades living cells. The cells of host plants grown in the manner described were somewhat vacuolate, yet quantities of protoplasm were not uncommon in invaded cells and even in contact with the hyphae (Plate II, B and C). Although haustorium-like bodies were noted and figured (Plate II, G), no true haustoria were observed.

After the initial hyphal entry, the fungus cells often were slightly smaller, of irregular shape, with very dense protoplasm containing few small vacuoles, and sometimes the septa were numerous (Plate II, E–G). It is, however, difficult to make any generalization, for the fungus was varied and irregular within the host tissues.

The spread of the fungus in the host usually occurred between the cells (Plate I, F, and III, B) with no visible evidence of cell-wall disintegration except at the place of entry. The hyphae branched freely and sometimes even completely surrounded a cell or cells (Plate II, H).

The fungus also advanced from cell to cell by passing directly through the cell walls (Plate III, A). In most cases the hyphae were constricted where they passed through the wall. Since these observations were made on young seedlings and the entrance of the fungus was in a small area just above the root cap, the spread was largely upward toward the stem (Plate III, B). In sections of roots of older infected seedlings grown in soil cultures the fungus appeared to spread in all directions from the place of entry. It also was found in the xylem of these roots, where it had advanced from one cell to another (Plate III, D). In no case in the seedlings grown on agar was there any visible differences noted between invaded and noninvaded cells.

Watermelon seeds (Iowa King variety) were surface sterilized by a 3-minute immersion in a 1:1,000 mercuric chloride solution. The seeds were then rinsed in sterile distilled water. Each seed was dropped with flamed forceps into a separate test-tube slant of potato-2 per cent dextrose-agar and placed in the incubator at a constant temperature of 28° C.. In 3 days all of the tubes were inoculated with the root-rot fungus except 4,

which were used as controls. All tubes were now kept on top of the incubator in the light in order to reduce etiolation as much as possible. The pure-culture inoculum was applied as a pseudo-sclerotium from a culture or as a piece of medium containing the fungus.

Hyphae from the inoculum, upon making contact with a root or rootlet, were noted to grow along the latter or to coil around it, meanwhile branching profusely. Examples were photographed (Plate I, A and C) and killed in Gilson's fluid to be used in cytological study. Pure cultures were isolated from the infected stems and roots.

The preceding experiment was repeated on Black-seeded Klondike, with almost identical results, except that the fungus was apparently more virulent on these seedlings that were killed when they had made very little growth. A fungus, identified microscopically as *Phymatotricum omnivorum*, was isolated repeatedly from the roots and stems of the seedlings.

The fungus was even more rapid and severe in its attack on Acala cotton seedlings grown in the same manner. Hyphae enveloped the roots very rapidly, then advanced up the stems onto the cotyledons, as was also noted in a few cases on the watermelon seedlings.

Eight-inch test tubes 1 inch in diameter were filled with soil from a root-rot area at the University farm near Tucson, Arizona. Water was added, the soil adjusted to water-holding capacity, and the tubes autoclaved for 2 hours at 15 pounds' pressure. Upon cooling, all tubes except controls were inoculated by placing a pure culture of *Phymatotrichum omnivorum*, grown on watermelon-rind plugs, underneath the surface of the soil. All tubes were placed in a constant-temperature incubator at 28° C. In 2 days mycelium was well distributed throughout the soil cultures, so watermelon seeds of Iowa King were surface-sterilized and planted, 2 in each tube. Prophylactic procedure obtained in all cases. Four days later, mycelium could be seen enveloping those rootlets growing against the inner surface of the tubes. The seedlings were now kept on top of the incubator where they received ample light. Sterile distilled water was applied at intervals to maintain the soil-moisture content. After 10 days the roots that could be observed where turning brown in places, and a binocular microscope showed them enveloped by abundant mycelium.

On the 25th day from planting 1 of the plants wilted and 6 more were beginning to wilt. The next day all 7 were dead. Seven days later all seedlings inoculated were dead, while the controls were in fine condition. Pure cultures of *Phymatotrichum omnivorum* were isolated from the diseased roots. Portions of the roots were placed in Gilson's fluid to be used in the study of the distribution of the parasite in the tissues.

A similar experiment was conducted, involving use of white sand instead of soil, and seedlings of both Iowa Belle and Iowa King were employed.

The results were variable. In general, the plants grown in soil cultures were most severely affected; in one case, however, large brownish lesions were found on the root of an Iowa Belle seedling 5 days after inoculum had been carefully placed near it. Iowa King seedlings, inoculated with a pure culture, were killed in 25 days (Plate I, A, a). Their roots had turned light brown and were enveloped with mycelium. The controls were in good condition (Plate I, A, b).

Effects of Mixed Cultures. In dealing with an aggressive and widespread soil-inhabiting parasite like *Phymatotrichum omnivorum*, its reactions in the presence of other fungi should be observed. From the standpoint of control the value of this information would be inestimable if some organism could be found that would definitely inhibit the growth and spread of the fungus without greatly impairing the normal growth of a host (9).

Neal (5) states that Aspergillus, Penicillium, and *Fusarium* spp. partially arrested the development of strands of *Phymatotrichum omnivorum* in agar plates simultaneously inoculated. Similar inhibiting qualities of the same fungi, as well as the depressing influences of various bacteria were noted in these studies.

In view of the work done by Weindling (26), a culture of *Trichoderma lignorum* was secured from Dr. H. S. Fawcett. Petri dishes of potato-2 per cent dextrose-agar, pH 7.2, were inoculated simultaneously on opposite sides with pure cultures of *Phymatotrichum omnivorum* and *T. lignorum*. The cultures were kept at a constant temperature of 28° C. Since the root-rot fungus grows more slowly than Trichoderma, the plates were inoculated with *P. omnivorum* on one side of the plate and after 3 days, when the fungus covered about one-third of the plate, the opposite side was inoculated with *T. lignorum*. The cultures grew directly toward each other without interruption. There was no evidence of any repelling influence of one fungus for the other. The fungi met in about 48 hours. Up to this time the mycelium of both cultures had grown very close to the culture medium, not projecting above its surface more than 3 mm. Where they contacted, some of the hyphae of each fungus grew straight toward the top of the Petri dish. At the same time most of the culture of *T. lignorum* grew over the top and in and around the root-rot fungus. On the other hand, the growth of *P. omnivorum* was almost completely stopped after the two cultures had encountered each other (Plate I, D, b). However, in a few cases the root-rot fungus grew luxuriantly along the roof of the Petri dish away from the mixture.

Wet mounds of aerial hyphae were examined at intervals. Several times a hypha of *Trichoderma lignorum* was found within a hypha of Phymatotrichum (Plate II, J). Evidently the former fungus obtains moisture and

nourishment from the root-rot fungus, for *P. omnivorum* was usually found to be very vacuolate and devoid of protoplasm when in association with Trichoderma. Furthermore, Trichoderma was found to twine and weave around strand hyphae of the root-rot fungus (Plate II, I).

To continue this investigation blocks approximately 1 cm. square of an 18-day-old culture on potato-2 per cent dextrose-agar permeated with both organisms were placed on agar slants. Precautions were taken to obtain blocks that were devoid of Trichoderma spores. In four days all tubes showed abundant growth of *T. lignorum*, but no growth of the root-rot fungus was evident. Even after 19 days *Phytomatotrichum omnivorum* had not grown from the inoculum. This indicates that the growth of the root-rot fungus is inhibited or killed by the presence of *T. lignorum*.

For an investigation of this nature to be of practical value, plants susceptible to root rot should be inoculated with a pure culture of Phymatotrichum and a duplicate set of plants with a mixture of the 2 fungi. This was done with Acala cotton and watermelon plants, but there are yet no results to report.

DISCUSSION

The data presented in these studies show that the watermelon, *Citrullus vulgaris*, is neither highly resistant nor immune, as was reported by Taubenhaus and Killough (25) and later by Taubenhaus and Ezekiel (24), but that it is susceptible. Field-grown plants of all 4 varieties, studied repeatedly, have been found infected with *Phymatotrichum omnivorum*. The different varieties vary in their susceptibility to the parasite and a detailed study of varietal susceptibility should be undertaken. In the field the roots of a few plants of the variety Iowa Belle apparently were infected by Phymatotrichum and the infected tissues were later walled off by the host, thus checking the spread of the fungus. In other cases the same variety was severely diseased. Thus it appears that the relative susceptibility may be variable within a given variety.

In general the entrance of the parasite into the watermelon roots, in the field, appears to have taken place in a manner similar to that described for cotton and alfalfa by Peltier, King, and Samson (12). This investigation, however, indicates that direct penetration by the hyphae is much more common and frequent than was heretofore recognized. The production of new roots just below the soil line, so common in cases of plants attacked by Texas root rot, was not observed on the watermelon. Although fungous filaments were abundant in the vessels of badly infected roots, no severe plugging was noted. It appears probable that the most important damage caused by the invasion of *Phymatotrichum omnivorum* is the destruction of cortical cells. This may interfere more with the passage of water from

the soil to the xylem rather than the conduction through the vessels of the xylem.

There appeared to be no repelling action of the host-cell cytoplasm for the fungus. The accompanying figures (Plate II, B, C and G) show that fungus filaments grew in direct contact with the cytoplasm. This suggests that *Phymatotrichum omnivorum* encountered no growth-inhibiting substances in the cells of the watermelon root. The fungus filaments were, however, often somewhat smaller when intracellular than when intercellular. The fact that *P. omnivorum* grew luxuriantly on watermelon-root, stem, and leaf media is further evidence that the plant is not a resistant one. Also, when small living watermelon fruits were inoculated the fungus grew and persisted.

Decrease in the size of the nuclei in invaded cells (compare Plate III, C and F with E) no doubt indicates that the normal function of the cells is being affected.

Although the seedlings used in the penetration studies were grown under somewhat abnormal conditions, the method employed assures the worker that he is dealing with pure cultures.

The manner in which the large-cell type of hyphae surrounded and enveloped a root, then sent one or more hyphae into the host, may be compared to a large appressorium-like structure. The fungus is in direct contact with the outside of the root; both mechanisms appear to aid the development of pressure to initiate penetration. The described softening action of penetrating hyphae is probably enzymatic in nature.

Results presented show that penetration is rapid in young seedlings. Evidence that few young plants in the field are killed by *Phymatotrichum omnivorum* may be due, at least in part, to the fact that the parasite may not be active early in the season when the soil is cool. Of course, there are other important factors to be considered. Why cotton seedlings grown in pots of inoculated soil do not succumb sooner than they do is not well understood.

Pure-culture studies show that *Phymatotrichum omnivorum* is inhibited or killed by the presence of *Trichoderma lignorum*. More work should be done on the effect of mixed cultures on *P. omnivorum*. This appears to be a promising field for the study of the control of Texas root rot.

SUMMARY

Watermelon, *Citrullus vulgaris*, has been repeatedly found susceptible to Texas (cotton) root rot in commercial planting in southern Arizona.

The causal organism, *Phymatotrichum omnivorum*, has been isolated from the roots of the dying watermelon vines, grown in pure culture, and has proved pathogenic on watermelon and cotton under sterile conditions

in the laboratory and in the field. All 4 varieties of watermelon tested were susceptible but there is need for further work to determine if any commercial varieties are resistant.

Cytological studies on the penetration of *Phymatotrichum omnivorum* into the roots of watermelon under pure culture conditions and field conditions show that the initial invasion of the host tissues is often a wedging of the hyphae between the host cells. Penetration of the host-cell walls appears to be due to pressure plus a softening of the cell wall at the point of contact, probably by enzymatic action.

The mycelium in the host tissue is either intra- or intercellular, but the latter condition usually is evident first. The individual cells are quite variable in size and shape and are often multinucleate.

The nuclei of invaded cells are much reduced in size and more dense than those of noninvaded cells.

Pure-culture studies show that *Phymatotrichum omnivorum* is inhibited or killed by the presence of *Trichoderma lignorum*. The possible effect of mixed cultures on the persistence of *P. omnivorum* in soils should be investigated.

UNIVERSITY OF ARIZONA,
TUCSON, ARIZONA.

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FUSARIUM YELLOWS OF CELERY[1]

T. C. Ryker

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INTRODUCTION

Fusarium yellows of celery[2] was first reported in 1914 by Coons (9) in the vicinity of Kalamazoo, Michigan, where it had been known to exist for 5 years before that time. It appeared in the field in small areas that became larger each succeeding year. Coons and Nelson (10) established the fact that the causal organism was a vascular Fusarium. Early attempts at control were founded upon the substitution of highly resistant varieties of the green type for those of the self-blanching type, but the limited market demand for the former required the abandonment of this method. In 1919 a breeding program was initiated at Michigan State College that resulted in the development of resistant strains of the self-blanching type (10, 20, 21). The disease has been reported in several northern States since 1914 (10). It was found in Wisconsin in 1931, when celery infected with the yellows organism was sent to the University from Green Bay. A badly diseased field was discovered near Waukesha the following year. The fact that the disease is gradually becoming more widespread indicates the possibility that it may assume serious proportions in the future in those localities where it has not yet gained a foothold.

The present paper is a report of studies of the symptoms of the disease, the pathogenicity and variability of the causal organism, the soil temperature and moisture relations of host and parasite, and the resistance of certain varieties of celery. An exhaustive taxonomic study of the parasite was not made, since this was already under way at Michigan State College. The several isolates used were compared with a culture furnished by Ray Nelson of that institution and were found to conform with the Michigan strain in their major morphological and cultural characters and in pathogenicity.

METHODS AND MATERIALS

Isolates of the celery-yellows organism were secured from 3 sources. Isolate 5 was obtained from M. E. Anderson of the Department of Plant Pathology, University of Wisconsin, who had isolated it from diseased celery plants sent to the University from Green Bay, Wisconsin, in 1931. Isolate 6 was received from Ray Nelson of Michigan State College, who

[1] The writer expresses his sincere appreciation to Dr. J. C. Walker for the valuable suggestions offered during the course of these investigations and for aid in preparation of the manuscript.

[2] This is not to be confused with the virosis of celery known as "yellows."

stated it to be one of his most virulent cultures. A number of isolations were made from diseased Golden Plume celery collected in a field at Waukesha, Wisconsin, in the summer of 1932. Isolates 14, 16, and 18 from this source were selected for study.

Varieties of the self-blanching type of celery on the whole are susceptible to yellows, while those of the green type are resistant. In the studies undertaken Golden Self-Blanching variety was employed as representative of the former type and Winter Queen of the latter. Seed lots of these varieties were obtained from the Olds Seed Company of Madison, Wisconsin. The following additional varieties were procured from the Grand Rapids Growers Inc., Grand Rapids, Michigan: Curly Leaf Easy Bleaching, a green type recommended for badly diseased fields; M. S. C. strain of dwarf Golden Self-Blanching, a yellows-resistant strain developed at Michigan State College, which, for the sake of brevity, will be referred to as Michigan G. S. B.; Michigan Golden, a strain of tall Golden Self-Blanching, also developed at Michigan State College and resistant to yellows. The last 2 varieties are reported to be resistant under field conditions in Michigan (21).

For the most part artificially infested soils were employed. A standardized method of inoculation was followed. One-quart Mason jars containing 300 grams of corn-meal-sand medium were autoclaved and a small quantity of spore suspension introduced. After the jars were incubated at room temperature for at least 2 weeks, their contents were incorporated in enough soil to fill a 6-inch soil-temperature-tank can, which held approximately 3,400 grams of soil. The soil employed consisted of a mixture of equal parts of sandy loam and muck. This mixture had a water-holding capacity of between 70 and 80 per cent. The naturally infested soil used was secured from a diseased field at Waukesha, Wisconsin.

Temperature and pathogenicity studies were carried out in the Wisconsin soil-temperature tanks described elsewhere (14). Plants were grown in galvanized iron cans of infested soil that were held at 18° C. or below for 5 days to allow the plants to recover from transplanting before they were placed in tanks at the desired temperature. In recording data, calculations were made from the time of planting rather than from the time the cans were placed in the tanks. The common air temperatures varied from 16° to 20° C. Soil moisture was held at 70 per cent of the water-holding capacity, unless otherwise designated.

Seedlings were grown in flats of yellows-free soil and transplanted when they had reached a height of about 4 inches. Plants were not recorded as diseased until the chlorotic symptoms appeared, since stunting, although often the first symptom was also caused by unfavorable growing conditions and might, therefore, be misleading.

SYMPTOMS OF FUSARIUM YELLOWS

The symptoms of celery yellows have been described by Coons (9) and Thomas (26); but, for the sake of clarity, they will again be reviewed in the light of the 2 seasons' observation on the part of the writer in a diseased field at Waukesha, Wisconsin, and in experimental trials in the greenhouse.

Stunting of the plants is the first visible symptom, which is quickly followed by a general chlorosis that begins with the larger veins of the leaves and progresses outward. This chlorosis is quite distinctive and usually is readily distinguished from chlorosis due to other causes. Often a slight downward curling of the leaflets precedes the chlorotic stage. The type of chlorosis induced under greenhouse conditions is similar to that induced in the field. Minor differences associated with different strains of the parasite will be discussed later in this paper. Occasionally, only a part of the plant shows chlorosis but, in such a case, all affected leaves are confined to the same side of the plant. Unilateral chlorosis of leaves sometimes occurs.

Concurrently with the chlorosis of the foliage, many of the roots reveal a general brownish discoloration and decay at the tips. The vascular tissue of the primary root usually shows a reddish brown discoloration, which extends into the stem and petioles as the disease progresses. Under field conditions the crown often splits and decays. This is probably due to secondary infection, for, under greenhouse conditions, it is not observed commonly. The plants gradually die with little wilting, except at high air temperatures. When older plants are affected, the symptoms appear first in the younger leaves. Such plants may remain alive throughout the season but their growth is decidedly stunted. Severity of the disease in the field is largely conditioned by the size of the plants and by the environmental conditions.

EXPERIMENTAL RESULTS

Pathogenicity of the Isolates

In testing the pathogenicity of the several cultures obtained, the gross cultures as isolated were used rather than monoconidial lines derived from them, for it was thought that by using the latter some potentialities of the fungus might be lost. Following the standardized procedure described, the pathogenicity of isolates 5, 6, 14, 16, and 18 was determined. Soils infested with the several cultures were planted with Golden Self-Blanching and Winter Queen celery and held at a temperature of 24° C. All isolates were pathogenic on Golden Self-Blanching, while Winter Queen was resistant in all instances. However, the degree of virulence and the symptom expression varied somewhat with the isolate. To establish further proof that the designated fungus was the causal agent, sections adjacent to pieces of roots

and stems that yielded the fungus upon plating were fixed and examined. The vessels showed the presence of numerous fungal strands. The reisolates were also pathogenic. The above studies indicated that the disease, under greenhouse conditions, manifests itself in a definite way that is not unlike its appearance under field conditions; namely, by the successive appearance of a slight downward curl of leaflets, by chlorosis, and death. Distinct wilting is uncommon.

The chlorosis was of 2 types, depending upon the cultures. Isolate 5 induced a general chlorosis of the entire foliage, which, on close examination, appeared as a yellowed flecking due to a clearing of the tissue in the vicinity of the veins and fine veinlets and to the fact that the small islands of leaf tissue subtended by the veinlets were in a state of progressive chloro-

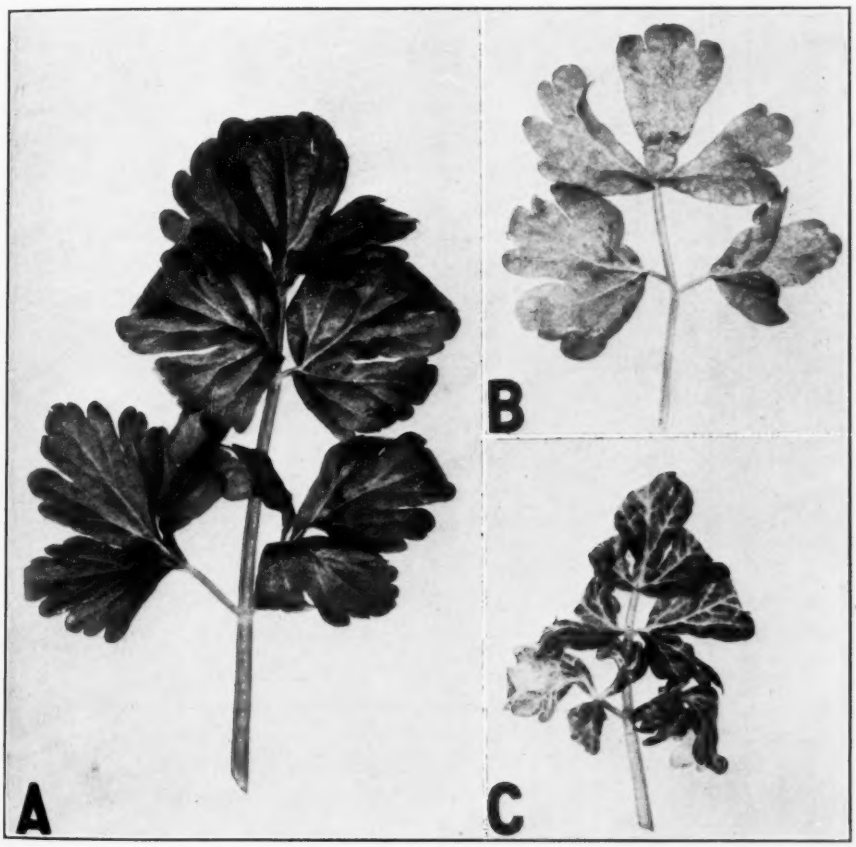

FIG. 1. Comparison of the 2 types of chlorosis produced on Golden Self-Blanching celery by different isolates. A. Leaf from a healthy plant. B. Leaf showing the general chlorosis induced by isolate 5. C. The type of chlorosis produced by the other isolates. In this type the initial chlorosis is confined to the larger veins.

TABLE 1.—*Development of yellows in Golden Self-Blanching and Michigan G. S. B. celery in soil infested with several isolates and in naturally infested soil held at temperatures of 24° and 28° C.*[a]

Soil temperature	Days after planting	Diseased plants in										
		Soil inoculated with										Naturally infested soil
		Isolate 5		Isolate 6		Isolate 14		Isolate 16		Isolate 18		
		Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden self-Blanching
° C.		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
24	22	0	0	55	17	75	0	33	0	25	17	0
	27	50	0	64	17	83	17	83	17	83	17	0
	32	92	0	100	33	83	50	83	67	92	17	0
	37	92	0	100	83	83	67	83	83	92	33	0
	43	92	0	100	83	83	67	83	83	92	33	17
28	22	0	0	90	0	83	100	75	67	67	0	0
	27	80	83	90	83	83	100	83	100	83	83	17
	32	90	100	90	83	83	100	100	100	92	100	33
	37	90	100	90	83	92	100	100	100	92	100	50
	43	100	100	100	100	92	100	100	100	100	100	83

[a] There were from 10 to 12 plants of Golden Self-Blanching and 6 of Michigan G. S. B. for each lot of soil at each temperature. The results from plants in noninfested soil are omitted, since they all remained healthy.

sis. This was in contrast to the chlorosis produced by the other isolates in which there was at first a clearing only in the region of the larger veins, while the remainder of the leaf tissue was green or only slightly yellowed. Eventually all tissue became chlorotic, although it was observed in several instances that plants died before this took place. The 2 types of chlorosis are illustrated in figure 1.

In the second experiment the relative virulence of the isolates upon Golden Self-Blanching and Michigan G. S. B. at soil temperatures of 24° and 28° C. was studied. Equal parts of soil were inoculated with each culture and planted to the 2 varieties. In addition, cans of naturally infested soil that had been diluted by one-half with a sandy loam were planted with Golden Self-Blanching. Plants of both varieties in noninfested soil served as controls (Table 1). The difference between the isolates in symptom expression was similar to that noted in the previous experiment. In this trial, isolate 5 was lower in virulence than isolate 6 or 14. It apparently takes isolate 5 longer to become established in the soil, since, in later trials with soil that had produced several crops of diseased plants after the original inoculation, this strain was as virulent as isolate 14 and more virulent than isolate 6.

The 2 varieties of celery reacted generally in the same manner under the conditions of the experiment. Disease inception at 24° C. was slightly slower in Michigan G. S. B. than in Golden Self-Blanching. The incubation period for Golden Self-Blanching in newly infested soil held at 24° was about 20 days. In other trials it was found that within certain limits the longer the soil had been infested the shorter was the incubation period. Disease development was more rapid in the artificially infested soils used than in the naturally infested one. This probably can be accounted for by the fact that a greater quantity of inoculum was present in the artificially infested soil.

Environmental Relations

The relation of environment to disease is best approached, insofar as is possible, through the study of a single environmental factor upon the parasite and the host separately and upon the interrelation of both in disease development. This method was used, particularly with temperature, in the study of celery yellows. The effect of temperature upon the growth of the causal organism was first considered. This was followed by a study of the effect of both soil temperature and soil moisture upon host development. The relation of these factors to the development of yellows was then investigated.

Relation of Temperature to Fungus Growth

Isolates 5, 6, 14, and 18, together with 4 monoconidial lines from isolate 14 and 2 monoconidial lines from isolate 16, were first grown on potato-

dextrose-agar plates. They were then transferred to fresh agar plates to be used for the temperature studies. Each culture of the fungus was thus prepared in triplicate for each of the following temperatures: 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. The diameter of each resulting colony was measured daily. All isolates reacted essentially alike. The absolute minimum and maximum temperatures were not determined, but the cultures showed slight growth at 8° and 36° C., good growth from 20° to 32° C., while the optimum fell close to 28° C. The data from isolate 14 is illustrated in graphic form in figure 2. The growth of isolate 5, 7 days after inoculation, is illustrated in figure 3. The average diameter of the colonies of any culture at all temperatures up to 32° C., when plotted against time, gave a straight-line curve, indicating that there was very little staling up to the time that the fungus had covered the surface of the Petri dish.

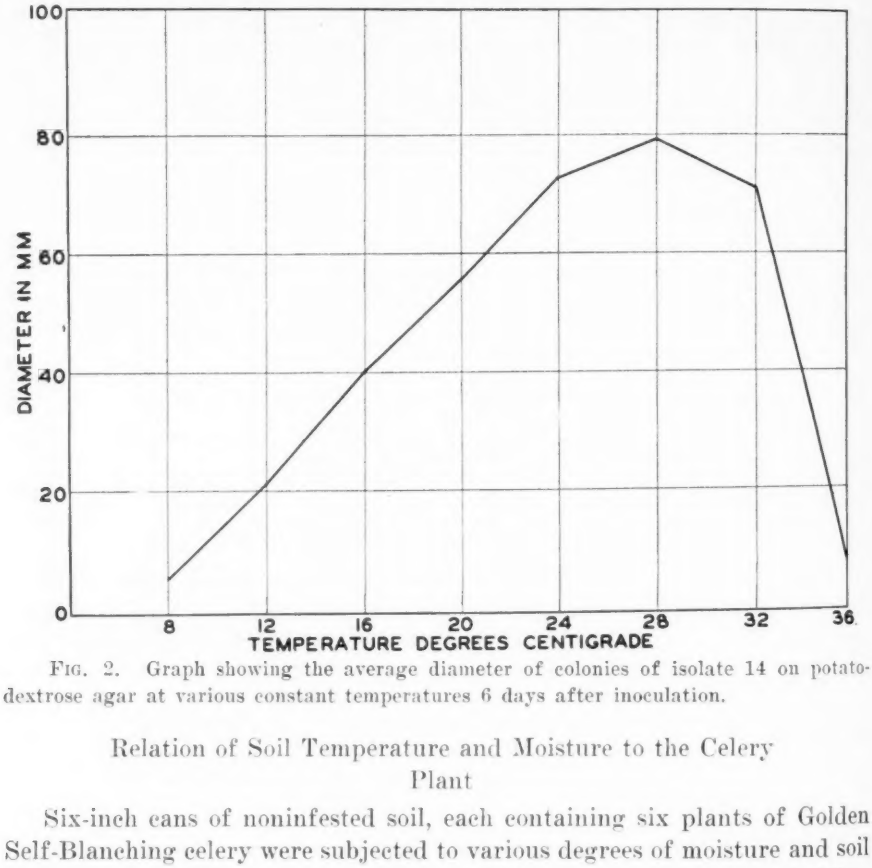

FIG. 2. Graph showing the average diameter of colonies of isolate 14 on potato-dextrose agar at various constant temperatures 6 days after inoculation.

Relation of Soil Temperature and Moisture to the Celery Plant

Six-inch cans of noninfested soil, each containing six plants of Golden Self-Blanching celery were subjected to various degrees of moisture and soil temperature. One can of soil, held at 50 per cent of its water-holding capacity and one at 70 per cent, were held at each of the following soil tem-

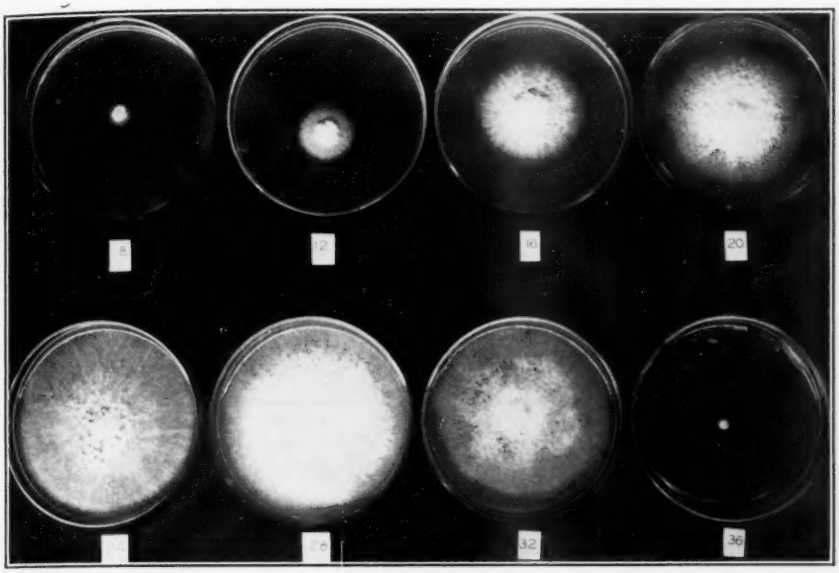

FIG. 3. Relation of temperature to the growth of isolate 5 on potato-dextrose agar. Representative colony at each of the designated temperature (°C.) 7 days after inoculation.

peratures: 18°, 20°, 22°, 24°, 26°, 28°, 30°, and 32° C. After 6 weeks the plants were removed and the soil washed from the roots. Measurements and dry weights are given in table 2. There was little difference in the growth of tops and roots at temperatures ranging from 18° to 28°, but at 30° and 32° distinct retardation occurred. This was shown not only by reduction in top height and the dry weights of tops and roots, but by chlorosis of the foliage and brown discoloration of the roots. When the plants at the 2 soil moistures are compared, it is shown that the best growth at any given temperature occurred in the higher soil moisture. The effect of low soil moisture was most severe at 30° and 32° C. Even higher moisture than 70 per cent of the water-holding capacity is tolerated by celery, since in other experiments where an occasional can leaked the plants grew well in nearly saturated soil.

Relation of Soil Temperature and Moisture to Development of Yellows

Preliminary studies on the effect of soil temperature showed that the critical low temperature for disease manifestation was somewhat below 20° C., while the optimum was around 28°. Further experiments will be reported in detail.

TABLE 2.—*Effect of soil temperature and moisture on the growth of Golden Self-Blanching celery*

Soil temperature	At 50 per cent of the water-holding capacity of the soil					At 70 per cent of the water-holding capacity of the soil				
	Plants	Tops		Average dry weight of roots	Ratio of dry weight of roots to dry weight of tops	Plants	Tops		Average dry weight of roots	Ratio of dry weight of roots to dry weight of tops
		Average height	Average dry weight				Average height	Average dry weight		
°C.	*Number*	*Cm.*	*Gm.*	*Gm.*		*Number*	*Cm.*	*Gm.*	*Gm.*	
18	5	15.2	.48	.15	1: 3.2	6	16.3	.92	.20	1: 4.5
20	6	12.8	.28	.08	1: 3.5	6	18.8	1.16	.19	1: 6.1
22	6	17.5	.68	.14	1: 4.9	6	18.7	.92	.15	1: 6.1
24	6	17.2	.66	.16	1: 4.1	6	20.2	1.19	.17	1: 7.0
26	6	15.7	.47	.09	1: 5.2	6	18.3	.80	.14	1: 5.7
28	6	16.7	.70	.12	1: 5.8	6	19.2	.99	.14	1: 7.0
30	6	9.8	.28	.05	1: 5.6	6	16.2	.70	.10	1: 7.0
32	4	8.0	.10	.03	1: 5.0	6	15.2	.66	.09	1: 7.3

In experiment 1 soil that had been inoculated some time previously with isolate 5 was placed in soil cans and planted with Golden Self-Blanching and Michigan G. S. B. celery. Three cans of infested soil for each variety were placed in soil-temperature tanks at each of the following temperatures: 18°, 20°, 24°, 26°, 28°, and 30° C. There were 7 plants to each can and the soil moisture was held at 65 per cent of the water-holding capacity. The degree of chlorosis, whether slight or marked, was recorded up to the time that plants began to die, after which time exact counts of diseased and dead plants were made (Table 3).

Yellows developed in Golden Self-Blanching at all temperatures. It appeared first at 28° and 30° where symptoms were markedly advanced at 15 days. It was progressively slower in appearance and rate of advance as the soil temperature lowered. At 18° it was not recorded until the 26th day, but by the 35th day 76 per cent of the plants were dead at this temperature. In later experiments, in which freshly inoculated soil was used, the incubation period was longer but the relative effects of the various temperatures were the same.

In the resistant variety, Michigan G. S. B., the effect of soil temperature was of the same order. This variety, however, exhibited distinct resistance at 18°, where no symptoms appeared. At progressively higher temperatures resistance decreased gradually until at 30° there was essentially no difference between the two except for the slightly slower rate of disease development in Michigan G. S. B.

In experiment 2 the soil used in the previous experiment was replanted with Golden Self-Blanching and Curly Leaf Easy-Bleaching celery and held at temperatures of 24°, 26°, 28°, 30°, 31°, and 32° C. The disease development in Golden Self-Blanching at 24°, 26°, and 28°, was similar to that in the preceding experiment except that symptoms appeared several days sooner. The disease was slightly more severe at 30° and 31° than at 28°, but development was slightly slower at 32°. At 30°, 31°, and 32° the control plants were stunted and somewhat chlorotic but the chlorosis was different in appearance from that induced by the fungus. Curly Leaf Easy-Bleaching plants remained healthy at 24° and 26° C. At 28° C. all plants were stunted and some showed typical disease symptoms, while at 30°, 31°, and 32° yellows was still more marked.

In experiment 3 the effect of soil temperature and soil moisture on the Golden Self-Blanching was studied, with 3 lots of soil infested with isolates 5, 6, and 14, respectively. One can of soil for each isolate at 50 per cent and one at 70 per cent of the water-holding capacity of the soil were used at the following soil temperatures: 18°, 22°, 24°, 26°, 28°, 30°, and 32° C. The results (Table 4) in the more moist soil were essentially like those cited in experiment 1 except that the disease was slower in appearing at 18° C.

TABLE 3.—*The effect of soil temperature upon development of yellows in Golden Self-Blanching and in Michigan G. S. B. celery*

Variety	Temperature	Total plants	After 15 days		After 20 days		After 26 days		After 35 days	
			Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
	°C.	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
	18	21	0	0	0	0	slight	0	91	76
	20	21	0	0	0	0	86	0	95	95
Golden	24	21	slight	0	95	0	95	95	100	95
Self-Blanching	26	21	do	0	100	91	100	95	100	95
	28	21	marked	0	100	86	100	95	100	95
	30	21	do	0	95	95	100	95	100	95
	18	21	0	0	0	0	0	0	0	0
	20	21	0	0	0	0	slight	0	slight	0
Michigan	24	21	slight	0	29	0	81	29	81	33
G. S. B.	26	21	do	0	14	0	100	29	100	86
	28	21	do	0	100	10	100	59	100	76
	30	21	do	0	100	24	100	86	100	91

(Table 4, Fig. 4). Isolate 6 was somewhat less virulent than the other 2 cultures. This difference in soil held at 20° C. is illustrated in figure 5, A. The vein chlorosis associated with isolates 6 and 14 and the general chlorosis associated with isolate 5 were consistent at all temperatures except 30° and 32° where chlorosis tended to become more diffuse with the first 2 strains.

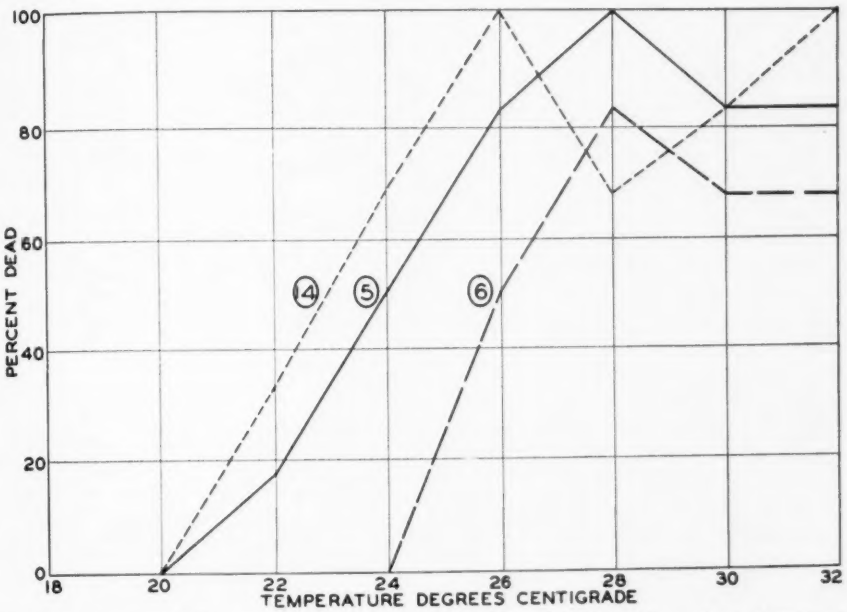

FIG. 4. Graph showing the percentage of Golden Self-Blanching plants killed with yellows after 28 days in soils infested with isolates 5, 6, and 14, respectively, and held at various constant temperatures and at 70 per cent of the water-holding capacity (Table 3).

With each isolate, disease development was decidedly slower in the dry than in the moist soil (Fig. 5, B). As noted above, the celery plant grows more slowly in the drier soil, and in plants thus stunted the disease always appeared more tardily than in vigorously growing individuals. This observation is similar to that of tomato wilt, in which Clayton (7) noted slower disease development in plants retarded by low soil moisture. The vein chlorosis in plants infected by isolates 6 and 14 was less distinct at low soil moisture.

The temperature range of the celery yellows organism is quite similar to the ranges of the other vascular Fusaria. Its growth curve follows closely that of disease development up to the optimum at about 28°, but above that temperature disease incidence does not drop off so abruptly as does radial expansion of the fungus. The disease develops at as high temperatures as

are favorable for the growth of the host. The minimum constant soil temperature for the disease was not determined, but at 18° it develops quite slowly. These results are in accord with the field observations of Nelson and Cochran (21), who reported that in the relatively warm summers of 1929, 1930, and 1931 susceptible varieties in Michigan were severely attacked, while during cooler summers the disease was of less importance.

FIG. 5. Golden Self-Blanching celery 1 month after transplanting. A. Left to right, noninfested soil, soil infested with isolates 5, 6, and 14, respectively. Note greater virulence of isolate 5. Soil held at 20° C. and 70 per cent of the water-holding capacity. B. Left to right, noninfested soil at 70 per cent water-holding capacity; same at 50 per cent water-holding capacity; soil infested with isolate 5 at 70 per cent water-holding capacity; same at 50 per cent water-holding capacity. All at soil temperature of 20° C. Note greater growth of the host and greater severity of the disease at the higher soil moisture.

VARIETAL RESISTANCE

Nelson and Cochran (21) list the celery varieties grown in Michigan in 4 groups, according to their susceptibility to yellows, as follows: *Very Susceptible*:—Golden Phenomenal, Hoover Special, tall strain of Golden Self-Blanching, and dwarf strain of Golden Self-Blanching; *Susceptible*:—Florida Golden and Golden Prize; *Moderately Resistant*:—Easy Blanching

TABLE 4.—*Development of yellows in Golden Self-Blanching celery in soil inoculated with isolates 5, 6, and 14 and held at various soil temperatures and moistures*

Soil temperature	Plants dead after 28 days[a]					
	Isolate 5		Isolate 6		Isolate 14	
	50 per cent w. h. c.[c]	70 per cent w. h. c.	50 per cent w. h. c.	70 per cent w. h. c.	50 per cent w. h. c.	70 per cent w. h. c.
° C.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
18	0	0	0	0	0	0
20	0	0	0	0	0	0
22	0	17	0	0	0	33
24	0	50	0	0	0	67
26	83	83	0	50	33	100
28	100	100	17	83	83	67
30	83	83	20[b]	67	80[b]	83
32	83	83	0	67	0	100

[a] 6 plants in each case except where indicated otherwise.

[b] 5 plants used.

[c] w. h. c. = water-holding capacity of the soil.

(Newark Market), Early Fortune, Golden Plume (Wonderful) and Golden Detroit; *Highly Resistant*:—Michigan Golden, and M. S. C. strain of dwarf Golden Self-Blanching. In the experiments already described it was shown that, as the soil temperature increased, resistance in Michigan G. S. B. and in Curly Leaf Easy-Bleaching became less pronounced. Golden Self-Blanching, was susceptible from 18° to 32°, although occasional plants remained healthy. Michigan G. S. B. was completely resistant at 18° but, as the temperature increased, typical yellows developed, although more slowly than in susceptible Golden Self-Blanching. Curly Leaf Easy-Bleaching remained free from symptoms up to 26° C. but developed typical yellows at 28° and above. Additional experiments confirmed these results. Winter Queen also was tested and reacted similarly to Curly Leaf Easy-Bleaching. Michigan Golden was grown in infested soil repeatedly at soil temperatures up to 31° and in no case was there any development of disease symptoms. This variety, therefore, appears to be distinctive in being completely resistant, even at high soil temperatures.

The above varieties were grown under various soil temperatures and platings were made from various plant parts to determine the relation of the fungus to the host. Golden Self-Blanching, at the time that symptoms

first appeared, showed the fungus to be most abundant in secondary roots, especially in those that were discolored. It was also abundant in the primary root, but became progressively less so as the stem region was approached, and it was found only rarely in the petioles. Michigan G. S. B. was similar to Golden Self-Blanching except that the fungus was rarely found in the primary root of plants grown at 18° and 20°. Curly Leaf Easy-Bleaching and Winter Queen showed the fungus abundant in secondary roots but only rarely in the primary root, at those temperatures below 28° where symptoms did not appear. However, in diseased plants grown at higher temperatures, the fungus was abundant in the primary roots. Michigan Golden, on the other hand, never yielded the fungus from the primary root but it was recovered from the secondary roots.

Histological examinations verified the above results. Golden Self-Blanching, Winter Queen and Michigan Golden were grown in infested soils held at temperatures of 24°, 26° and 28° C. Plants were removed 30 days after planting and portions were fixed, embedded, and stained. At this time Golden Self-Blanching plants were diseased at all temperatures, Winter Queen only at 28° C. and Michigan Golden at none of these temperatures. In Golden Self-Blanching the fungus was abundant in the cortical region of the secondary roots and in the vessels of both secondary and primary roots. Instances were rare of the passage of the fungus from the cortical tissue into the stelar portions. Apparently, the inner cortical cells of older roots act as a barrier even in a susceptible variety. Within the stele the fungus was confined principally to the xylem vessels where it occurred in some instances in sufficient quantity to cause complete plugging. Occasionally, in secondary roots, the tissue adjacent to the vessels had collapsed and had been penetrated by the fungus. This condition was rare in the primary roots. With the extension of the vessels into the stem, fewer hyphae were noted and in most instances they did not occur beyond the transition region. They were only rarely observed in vascular tissue of the petioles.

Winter Queen, at temperatures that did not induce disease symptoms, showed the fungus abundant in the cortex and rare in the xylem of secondary roots. Diseased plants of this variety showed the fungus as widely distributed as in Golden Self-Blanching. In Michigan Golden the fungus was present in the cortex of secondary roots but never in the stele. The relation of the fungus to the cortical cells of all 3 varieties was essentially the same, the fungus being either inter- or intracellular.

The above results may be compared with those secured by others in varieties of cabbage resistant to *Fusarium conglutinans* Wr. Anderson (1) found in the Wisconsin Hollander cabbage that, with the rise in soil temperature, increasing percentages of diseased plants appeared until, at

24° C., resistance usually disappeared entirely. This variety is somewhat analogous to Michigan G. S. B. celery in that resistance is confined to relatively low soil temperatures. Walker and Smith (28) showed that in homozygous resistant lines of cabbage at soil temperatures of 26° and above, symptoms were produced but they were atypical and the fungus was not isolated from the tissue. The situation is somewhat different in celery yellows, in that those varieties that become diseased only at soil temperatures of 28° C. and above, such as Winter Queen and Curly Leaf Easy-Bleaching, develop typical symptoms and the fungus is present in the host tissue. Michigan Golden is most comparable to homozygous resistant cabbage, since it is completely resistant at all temperatures and fungus invasion is confined to the outer cortical cells of secondary roots.

VARIABILITY OF THE PARASITE

It has been shown already that the several isolates varied in their degree of virulence and in the type of symptom produced upon the host. Further studies were made with isolates 5, 14, and 16, which at the time had been carried in pure culture for 2 years or more. The range of variation in cultural characters was investigated first, followed by studies in pathogenicity. Monoconidial lines were isolated following the method described by Keitt (16). Microconidia were used since cytological studies indicated that they were all uninucleate. Cultural characters were studied on potato-dextrose agar that had been thoroughly mixed before pouring. A single lot of medium was used for each series.

In the first series of monoconidial cultures a wide range of variability was noted in those derived from isolates 5 and 16, while those from isolate 14 were very similar to one another. The 31 cultures derived from isolate 5 were placed in 5 groups and the 22 lines from isolate 16 were placed in 3 groups. Thus 9 groups were distinguished from one another on the basis of the kind and amount of aerial mycelium, the type of subaerial mycelium, and the degree of pigmentation of the substrate. Two cultures were selected from each distinct group and 10 conidia were isolated from each. This procedure was continued for 4 conidial generations. Thus about 750 microconidia were isolated and cultured and their thalli studied. There was a tendency toward stability in cultural characters, although some segregation continued to occur in some of the lines.

Nine monoconidial lines that showed the highest degree of constancy were then selected as representative of the various culture types. Ten monoconidial subcultures were made from each and this procedure was repeated through 10 successive conidial generations. Eight of the lines remained constant in culture type. One line segregated in the fifth conidial generation. The divergent form remained constant through the five suc-

ceeding generations. The growth rate of all these cultures on potato-dextrose agar was essentially the same at 24° C.

The 9 original cultures just mentioned were tested for pathogenicity at once and again after they had remained in culture tubes for about 2 months. The most susceptible variety, Golden Self-Blanching, and the most resistant variety, Michigan Golden, were included in the pathogenicity tests. The latter remained free from symptoms in all cases, while the disease developed as usual on the former. All of the cultures derived from isolate 5 produced the general chlorosis typical of that isolate, while those derived from the other isolates produced symptoms characteristic of their respective sources. It was evident that this property was very well fixed in the respective isolates. The 9 original lines varied from high to low virulence. This was consistent in each of 3 pathogenicity trials. The relative virulence of the 9 original cultures is illustrated in figures 6 and 7.

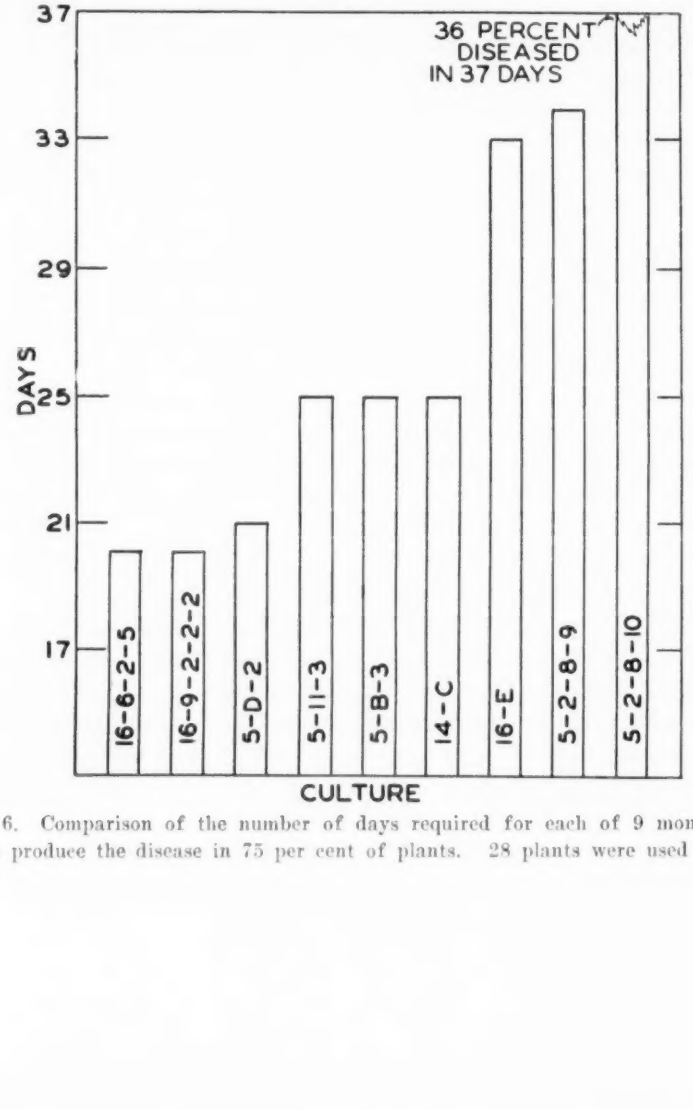

FIG. 6. Comparison of the number of days required for each of 9 monoconidial strains to produce the disease in 75 per cent of plants. 28 plants were used for each culture.

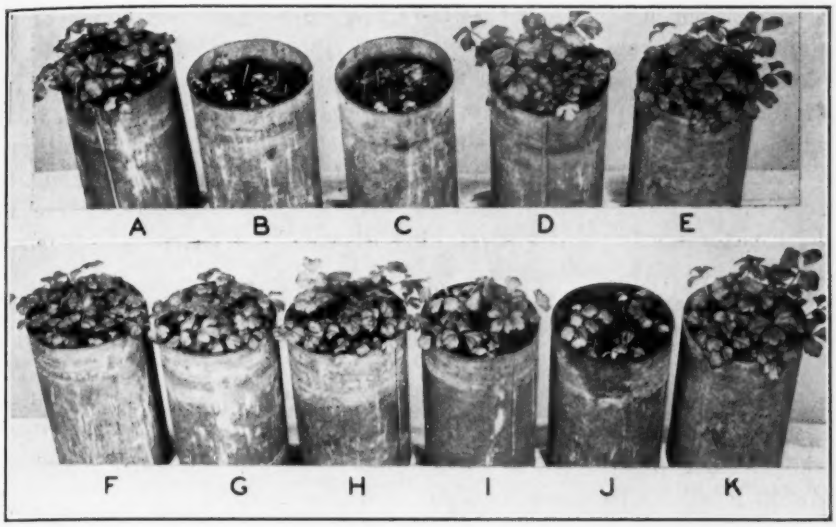

FIG. 7. Comparison of the relative virulence of 9 monoconidial lines derived from 3 original isolates of the celery-yellows organism on Golden Self-Blanching celery, 39 days after planting in soil at 25° C. A was derived from isolate 14; B, C, D were derived from isolate 16; F, G, H, I, J were derived from isolate 5; E and K are controls in noninfested soil.

Reisolations were made from plants infected with each strain. In all cases but one the reisolate was similar in cultural characters to the original. In the exceptional case one reisolate produced pigment in the substrate while the other, like the original, did not. Three reisolates from low-virulent strains and 2 from high-virulent strains were subcultured. Seventy-four subcultures, thus secured, were tested for pathogenicity on Golden Self-Blanching. These showed a wide range in degree of virulence and there was no correlation with the virulence of the mother culture nor with the type of cultural character.

It may thus be concluded that there is a tendency for the monoconidial lines to approach a certain degree of constancy in cultural characters through repeated single-spore selection. This is not true with regard to degree of virulence on Golden Self-Blanching variety. Two pathogenic characters, however, did not vary. The type of symptom on the susceptible variety remained constant and the complete lack of ability of all the isolates to produce symptoms on Michigan Golden appeared to be fixed.

DISCUSSION

Soil temperature has been shown to be an important influential factor in a number of diseases caused by vascular Fusaria (2, 8, 15, 19, 27). It

has been shown in this study that the disease developed very slowly at 18° C., and that the rate increased with temperature up to 28° C. Thus the temperature curve for growth of the organism followed closely that for disease development. The influence of temperature upon the disease, therefore, appeared to result from its effect upon the parasite rather than upon the host. Soil moisture, on the other hand, affected disease development through its influence upon the host. At low soil moisture, where the growth of the celery plant was retarded, disease development was decidedly slower than at high soil moisture, which favored the host.

Soil temperature also affected the expression of resistance in certain varieties. Michigan G. S. B. was highly resistant only at 18° C., while Winter Queen and Curly Leaf Easy-Bleaching were resistant up to 26°. This indicates that the resistant principle does not function at certain soil temperatures and that the temperature range for resistance varies with the variety. Michigan Golden is outstanding in that it remained completely resistant at all temperatures to which it was exposed.

That variability occurs in the celery yellows parasite is not surprising, for it has been shown to be characteristic of a number of vascular Fusaria (5, 22, 23, 29). The variability in this group has been recognized by differences in cultural characters and in degree of virulence. It has been shown in these studies that the extent of variability in a very limited number of isolates was quite great and extended into successive generations of monoconidial cultures derived from the isolates. These differences were expressed in cultural characters, in degree of virulence, and in the type of symptom produced. It was further shown that after continued subculturing certain lines showed a marked degree of uniformity in cultural characters and that, once purified, passage through the host produced little change in most of them. This did not preclude the possibility of changes in virulence. The study of the pathogenicity of a large number of monoconidial cultures from reisolates of low- and high-virulent strains indicated that the subcultures varied considerably in virulence. The parent reisolates maintained their relative standings in this regard, although there were indications that as a result of their passage through the host they had become somewhat more virulent, particularly so in the case of the reisolates of strains of low pathogenicity. If the cultures be inconstant in virulence, then it would be expected that the reisolate would tend to show the upper virulence range of this fluctuation. On the other hand, if the same strains be grown on artificial media, the chances of perpetuating either low or highly virulent forms are equal. The apparently constant fluctuation between higher and lower virulence would suggest that it would be but a step from a low virulent form to an entirely saprophytic one, or, conversely, from a nonparasitic to a parasitic state. This might indicate that the

vascular Fusaria arose as one or more groups and that, through the fluctuation of certain of its members, forms developed that were parasitic to a specific host.

The results of these studies would further suggest that any taxonomic study of the parasite must take into consideration not only the variability in a single isolate but that of a great many taken from a wide geographical range. No evidence of selective host pathogenicity was evinced in these studies, but this cannot be regarded as conclusive of its absence until more extensive studies have been made, for, since the parasite can be sufficiently divergent to produce different symptom expressions on the same host plant, it might well extend to a differential in host specialization between strains.

The reaction of these variants raises the question of the mechanism involved. Theories have been proposed to explain variability but little real evidence has been advanced as to its cause. Leonian (17, 18) disposed of the necessity of explaining the mechanism by considering dissociation a phenomenon whereby the variability of the culture traces the limits of the species and is thus a normal behavior. This may or may not hold true of the parasite with which this paper deals.

Brierley (3, 4) considered that dissociation could be accounted for by a heterocaryotic condition of the original isolate, which he believed to be followed by a reassortment of nuclei in the variants. More recently, Hansen and Smith (12) have presented experimental data in support of this theory They believed that hyphal fusions brought about the heterocaryotic condition. Leonian (18), however, was not able to induce "Mixochimaera" in *Fusarium moniliforme* Sheldon by mixing different cultures. The variability of celery Fusarium was not of this order, for cytological study indicated that the microconidia were uninucleate. This was supported by evidence not incorporated in the above studies in which attempts were made to hybridize 2 lines of fixed culture types by mixing spore suspensions and growing the resultant cultures on plates and tube slants. Only the 2 parent types could be reisolated.

Several workers are of the opinion that certain variations shown are due to mutation (24, 25). However, the extent to which variation in this fungus occurs would necessitate the assumption that chromosome aberrations occur frequently. Caldis and Coons (6) found that more or less permanent changes could be induced by certain changes in environmental conditions, but these were negated by passing the organism back through the host. This induced variation they believed to be similar to the *Dauermodification* of Jollos (13). This would not seem to be the case with the celery Fusarium, however, for, by passing the fungus back through the host, few changes in culture type were induced. The delayed segregation of Dickinson (11) is likewise not tenable, since the sexual stage has never been

observed in any of the vascular Fusaria. Thus it appears to remain for still another explanation to account for the variability exhibited by the celery-yellows organism.

SUMMARY

This paper reports the results of studies upon the pathogenicity, the environmental relations, and the cultural and pathogenic variability of the celery-yellows organism.

The symptoms of celery yellows were quite distinct and agreed with those described by other workers. In greenhouse studies 2 types of chlorosis were distinguished, depending upon the isolate used.

Isolates obtained from 2 localities in Wisconsin agreed in pathogenicity with one obtained from Michigan but there were differences in the degree of virulence and in certain culture characters.

All isolates had essentially the same temperature relations when grown on potato-dextrose agar held at various constant temperatures. The extremes of growth were close to 8° and 36° C., good growth from 20° to 32° C. and the optimum was at about 28° C.

The celery plant is favored by soil temperature of 18° to 28° C. above which there is marked stunting.

Disease developed in the susceptible Golden Self-Bleaching variety at soil temperatures of 18° to 32° C. the latter temperature being close to the limit of host tolerance. The incubation period was shortened proportionately with the increase of temperature up to 28° C.

Soil temperature affected the resistance of certain varieties. Michigan G. S. B. was highly resistant only at 18° C., Winter Queen and Curly Leaf Easy-Bleaching up to 26°, while Michigan Golden was resistant at all temperatures studied. All varieties, whether diseased or not, showed fungus present in the cortical region of secondary roots, but only plants showing symptoms contained it in the vessels of the primary root.

At low soil moisture, where host development was retarded, the development of the disease was checked proportionately.

Monoconidial cultures of several isolates varied in cultural characters and in the degree of virulence. Continued subculturing tended to produce constancy in culture characters. On passing these through the host the cultures, for the most part, remained unchanged.

Virulence was not fixed by continued subculturing.

The type of symptoms produced remained constant in the various isolates, and all strains of the fungus failed to produce the disease in Michigan Golden variety of celery.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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QUANTITATIVE STUDIES ON THE FILTRATION OF TOBACCO-MOSAIC VIRUS[1, 2]

H. H. THORNBERRY

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INTRODUCTION

Previous investigations on the filterability of plant viruses have been either qualitative in nature or the quantity of virus in the filtrate was determined from the highest dilution that remained infectious. With the bean local-lesion method of determining the concentration of samples of tobacco-mosaic virus, developed by Price (24), dilution to the end point of infectivity is not necessary for measuring the amount of virus present in a given sample, since the number of lesions produced on the bean leaves depends on virus concentration. The method, therefore, allows a more rapid as well as more accurate quantitative study of the filterability of tobacco-mosaic virus than has been possible previously. The purpose of the studies reported in this paper was to determine the amount of tobacco-mosaic virus that passes filters of different kinds under various conditions and to estimate by ultrafiltration the size of virus particles.

REVIEW OF LITERATURE

In 1892 Iwanowski (16) obtained typical mosaic symptoms in tobacco plants inoculated with infectious juice that had passed bacteria-proof filters. He believed that a toxin secreted by a causal bacterium in diseased plants passed into his filtrate and produced the symptoms of disease. Beijerinck (5), in 1898, also obtained typical mosaic symptoms in healthy plants inoculated with a bacteria-free filtrate (Chamberland filter) of infectious juice. By successive subinoculations he proved that the disease was not caused by a filterable nonmultiplying toxin, as Iwanoski believed, but by a filterable multiplying agent, which he termed *Contagium vivum fluidum*.

Some of the virus diseases of plants reported to be produced from bacteria-free filtrates are:

Tobacco mosaic—Iwanoski (16), 1892;
Cucumber mosaic—Doolittle (8), 1920;
Healthy potato—Johnson (18), 1925;
Streak of tomato—Vanterpool (32), 1926;
Aucuba mosaic—Smith (26), 1928;

[1] Presented as part of a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy; granted June 18, 1934.

[2] Published at the expense of The Rockefeller Institute for Medical Research out of the regular order of date of acceptance of the manuscript.

Curly top of sugar beet—Severin and Swezy (25), 1928;
Potato mosaic—Smith (27), 1928;
Sisal disease—Staner (29), 1929;
Ring spot of potato—Smith (28), 1929;
Yellow dwarf of onion—Melhus *et al.* (22), 1929;
Glasshouse streak of tomato—Jarrett (17), 1930;
Rose wilt and dieback—Grieve (12), 1931;
Ring spot disease of tobacco—Henderson and Wingard (15), 1931;
Hyoscyamus IV—Hamilton (13), 1932;
Streak disease of maize—Storey (30), 1932.

Filterability of tobacco-mosaic virus has been verified by several investigators, but only Chapman (7) has reported quantitative studies. On the basis of dilution capacity of the filtered and nonfiltered samples, he stated that Chamberland (grade not mentioned), Berkefeld "N," Berkefeld "W," and Kitasato filters allowed 91, 63, 47, and 40 per cent, respectively, of the virus to pass.

METHODS

Samples of tobacco-mosaic virus were obtained from plants of *Nicotiana tabacum* L. var. Turkish that had been kept in a greenhouse for about a month after infection with Johnson's virus No. 1. After harvesting and macerating the plants in a food chopper, the infectious juice was strained through double thickness of cheesecloth under pressure of wringing by the hands, placed in test tubes, and frozen immediately. Subsequently, it was thawed and refrozen on 2 occasions before being stored in an ice-box. This treatment did not noticeably affect infectivity of the virus, but cleared the infectious juice of coarse plant-tissue fragments.

Filters employed were Berkefeld candles ("V," "N," and "W"), coarse, medium, and fine; Seitz E. K. filter pads; Kramer's plaster-of-Paris and magnesium oxide filters; Jena sintered-glass filters; Bechhold-Konig filters; and collodion ultrafilters. The Berkefeld candles were used repeatedly. Between filtration experiments they were cleaned by filtering 100 cc. of boiling sodium carbonate solution (2 per cent), followed with 50 cc. of potassium permanganate solution (10 per cent), then with 50 cc. of a mixture of equal parts of hydrochloric acid (5 per cent) and sodium sulphite solution (10 per cent). After passing 200 cc. of boiling distilled water through each filter, it was sterilized by autoclaving. The other filters were used only once.

Testing of filters included the determination of pore size, imperviousness to bacteria, and the sign of the electrical charge on the filtering surfaces. The size of pores in the Berkefeld, Jena sintered-glass, Bechhold-Konig filters, and collodion ultrafilters, was determined by Bechhold's (3) air-water method. Faulty or broken filters were easily detected by the size of the bubbles that arose from the candle and by the pressure required to

force air through the pores. Filters that proved to be satisfactory by this method were further tested for their imperviousness to *Bacillus prodigiosus* Flügge. This was accomplished by filtering a suspension of the organism through the filters and plating out aseptically a given volume of the filtrate. Absence of bacterial colonies in the plates after 4 days' incubation was evidence that the filters retained the bacteria.

The sign of the electrical charge on the surface of filters was determined by filtering solutions of acid and basic dyes. Filters possessing a negative charge retain basic dyes (colored cation) because of the adsorption of positive ions to the negative surface of the filter. Those possessing a positive charge retain acid dyes (colored anion) by adsorption, while those with both negatively and positively charged areas retain both dyes. It was found in these determinations that Berkefeld candles, Seitz E. K. filter pads, and collodion ultrafilters retained Victoria Blue (basic dye) between pH 1.5 and pH 8.5, and allowed Congo Red (acid dye) between pH 5.0 and pH 8.5 to pass through. Therefore, these filters are negatively charged with respect to the dyes through the pH range at which virus suspensions (pH 1.5 to 8.5) were filtered. Kramer's plaster-of-Paris filters prepared for these studies retained both dyes. The Jena sintered-glass and Bechhold-Konig filters, due to their coarseness, allowed both dyes to pass through.

Negative pressure of 700 mm. of mercury was used throughout, unless otherwise stated. It was maintained by means of a glass water pump and was measured with a mercury manometer. Filtration was not started until the negative pressure reached the desired value.

Virus concentration was determined quantitatively by the bean local-lesion method described by Price (24). By this method infectivity of the virus is measured, and the approximate amount of virus in a given sample may be determined from the number of lesions produced. Since infectivity was found in other experiments to be optimum at pH 7.0 to 8.5 in 0.1 molar phosphate buffer, all the virus suspensions, unless otherwise stated, were adjusted to pH 8.5 in 0.1 molar disodium phosphate before inoculation. Immediately after inoculation, the plants were washed with running tap water and kept in a greenhouse, under conditions favorable for growth, until local lesions became conspicuous enough (4 or 5 days) for counting. Due to the difficulty of counting lesions in excess of 300 per leaf, the samples that were known from previous experience to contain abundance of virus were diluted before being used for inoculating beans. This was justified, since the number of lesions produced on a leaf was found in other experiments to be proportional to the virus concentration when the original virus suspension was diluted 1×10^{-4} or more in 0.1 molar disodium phosphate. The values for the concentrated virus samples given in the tables, therefore, constitute the number of lesions multiplied by the dilution value

of the virus suspension. Inoculations were made by rubbing the upper surface of the primary leaves with a bandage gauze pad previously moistened with 5 cc. of the virus suspension to be tested. All the samples of virus in a given experiment were inoculated as soon as possible to 12-day-old beans of the variety Scotia.

EXPERIMENTATION

Filtration of Virus Diluted in Distilled Water

The amount of virus in water dilutions that passes through Berkefeld "W" candles was determined by filtering 100 cc. of each dilution and measuring the virus activity of the filtered and unfiltered samples. The suspensions were applied to beans without adjusting the reaction, which varied from pH 5.6 to 6.8 as determined colorimetrically. The results are given in table 1.

TABLE 1.—*Number of lesions produced by Berkefeld "W" filtrates and by non-filtered samples of tobacco-mosaic virus at various dilutions in distilled water*

Virus dilution	pH of medium	Times repeated	Number of local lesions[a]		Virus activity in the filtrate
			Unfiltered	Filtered	
					per cent
2×10^{-1}	5.6	10	1932 ± 91.05	1532 ± 80.26	79
1×10^{-1}	5.8	10	1889 ± 163.90	1368 ± 120.73	72
4×10^{-1}	6.0	10	1479 ± 89.03	1299 ± 68.79	87
$.2 \times 10^{-1}$	6.0	10	1355 ± 103.87	1174 ± 64.07	86
1×10^{-2}	6.2	10	748 ± 24.28	694 ± 44.51	92
$.2 \times 10^{-2}$	6.4	10	518 ± 37.09	515 ± 30.35	99
1×10^{-3}	6.5	10	335 ± 32.37	387 ± 43.84	115
Check			0		

[a] = Average number of lesions on 20 beans.
± = Probable error.

These measurements show that the Berkefeld "W" filtrate was at least 75 per cent as infectious as the unfiltered sample. They also show that a greater percentage of the virus filters through at high dilutions than at low dilutions. At 1×10^{-3} dilution the filtered suspension was 15 per cent more infectious than the unfiltered. This suggests that filtration may disperse the virus or remove an inhibitory substance and thus result in an increased number of active infective units. Because of the effect of filtration on virus infectivity (conclusively shown later in this paper), the quantity of virus in filtered samples as compared with the quantity in unfiltered samples is not determined accurately by the local-lesion method. The number of lesions produced from a given sample, however, depends upon the approximate amount of virus in the suspension.

Filtration of Virus at Various Hydrogen-ion Concentrations

The amount of virus passing through Berkefeld "W" candles at reactions of various pH values was determined in the following experiments. Virus (100 cc.) at a 1×10^{-1} dilution in 0.1 molar phosphate buffer (pH 8.5, 5.6, 4.5, 3.0, and 1.5) and in distilled water (pH 5.6) was filtered. The residue was resuspended in 0.1 molar disodium phosphate. Before estimating virus infectivity in the various samples by inoculating beans, the reaction was adjusted to pH 8.5 in 0.1 molar phosphate buffer. Results of these measurements are given in table 2.

TABLE 2.—*Filterability of tobacco-mosaic virus at reactions of various pH values through Berkfeld "W" candles*

Virus suspension	Unfiltered	Filtrate	Residue
Buffered at pH 8.5	85,890[a] ±	142,356 ±	2,324 ±
	5,925.2	8,183.70	647.3
Without buffer pH 5.6	74,113 ±	32,797 ±	108,853 ±
	13,131.4	1,137.20	2,230.5
Buffered at pH 5.6	105,300 ±	25,953 ±	92,142 ±
	4,241.7	2,687.20	1,350.1
Buffered at pH 4.5	64,550 ±	30.7 ±	104,958 ±
	646.9	2.99	986.4
Buffered at pH 3.0	121,500 ±	2.3 ±	126,937 ±
	7,756.7	.337	4,562.5
Buffered at pH 1.5	134,000 ±	0.0	106,032 ±
	6,340.3		6,205.3
Check	0		

[a] = Average number of local lesions on 20 beans.
± = Probable error.

These data show that the reaction of the suspension determines the amount of virus passing through Berkefeld "W" candles. At pH 8.5 the filtered virus was 66 per cent more infectious than the unfiltered. This increase was probably due to virus dispersion and not to the removal of an inhibiting substance, since the residue left on the filter had no detectable effect upon the infectivity of added virus. The fact that the residue was almost inactive indicated that nearly all of the virus filtered through at this reaction. A part of the virus passed through in acid media of pH 5.6, 4.5, and 3.0. According to these data, the filtrates were 44.2 and 24.6 per cent as infectious as the unfiltered juice at pH 5.6 in buffered and unbuffered suspensions, respectively, while at pH 4.5 and 3.0 they were 0.047 and 0.0019 per cent as infectious. Virus was not demonstrated in the filtrates at pH 1.5. Nonfilterable virus in the residue was recovered by elution in 0.1 molar disodium phosphate.

Fractional Filtration of Virus through Berkefeld "W" Candles

The amount of virus in various portions of the filtrate was determined by fractionation experiments in which virus (100 cc.) at a 1×10^{-1} dilution in 0.1 molar phosphate buffer (pH 8.5, 5.6, 4.5, 3.0, and 1.5) and in distilled water (pH 5.6) was filtered through Berkefeld "W" candles. The filtrates were fractionated into 10 portions of 10 cc. each as the liquid passed through the candles. Results of the measured virus infectivity of the various fractions are given in table 3.

TABLE 3.—*Virus infectivity of successive 10 cc. fractions of Berkefeld "W" filtrate of tobacco-mosaic virus at reactions of various pH values*

Fraction	Average number of lesions on 20 beans					
	Without buffer	With 0.1 M phosphate buffer				
	pH 5.6	pH 8.5	pH 5.6	pH 4.5	pH 3.0	pH 1.5
1st 10 cc.	8,882 ± 1,127.7	142,540 ± 13,073.7	16,850 ± 1,922.3	70 ± 22.3	4 ± 1.34	0
2nd 10 cc.	31,280 ± 2,918.8	145,340 ± 15,843.7	30,900 ± 3.844.6	80 ± 13.4	2 ± 1.34	0
3rd 10 cc.	41,380 ± 4,948.6	141,740 ± 12,787.3	32,800 ± 5,935.6	60 ± 26.9	4 ± 1.34	0
4th 10 cc.	50,380 ± 7,767.6	138,000 ± 5,765.8	43,000 ± 1,618.8	80 ± 0.0	3 ± .67	0
5th 10 cc.	98,960 ± 6,639.8	137,100 ± 10,713.5	84,200 ± 2,832.9	35 ± 3.3	4 ± 2.69	0
6th 10 cc.	23,962 ± 1,997.3	149,740 ± 7,932.5	15,980 ± 2,778.9	30 ± 6.7	3 ± .67	0
7th 10 cc.	25,386 ± 1,398.7	148,140 ± 7,600.0	9,860 ± 1,270.6	15 ± 11.3	3 ± 2.02	0
8th 10 cc.	21,182 ± 1,315.8	138,540 ± 6,731.1	9,180 ± 925.6	0	0	0
9th 10 cc.	17,122 ± 1,152.7	146,600 ± 19,623.5	9,820 ± 2,751.9	0	0	0
10th 10 cc.	6,257 ± 1,755.5	165,020 ± 11,735.4	6,940 ± 1,829.9	0	0	0
Unfiltered	74,133 ± 13,131.4	85,890 ± 5,925.2	105,300 ± 4,241.7	64,550 ± 646.9	121,500 ± 7,756.7	134,000 ± 6,340.3
Check	0	0	0	0	0	0

± = Probable error.

It is evident from these data that essentially all of the virus passed through the filters at pH 8.5, both at the beginning of filtration and thereafter. The filtered fractions were from 47 to 92 per cent more infectious than the unfiltered juice. Percentage infectivity of virus in the 10 filtered

fractions, compared with the infectivity of the nonfiltered juice, was 165.0, 169.2, 165.1, 149.0, 147.9, 174.4, 149.2, 161.2, 171.6, and 192.1, respectively.

A small amount of virus at pH 5.6 passed through the candles at the beginning of filtration. As filtration proceeded, the filtrate became more infectious until 50 cc. had filtered through when the infectivity of the 6th fraction was equal to that of nonfiltered juice. Later fractions proved to be less than 50 per cent as infectious as nonfiltered juice. Infectivity of the 10 fractions of the nonbuffered filtrates was 11.9, 42.2, 55.8, 67.9, 137.8, 32.3, 34.2, 27.2, 23.0, and 8.4 per cent of that of the nonfiltered virus, and 16.0, 29.3, 31.1, 40.7, 79.8, 15.1, 9.3, 8.7, 9.3, and 6.5 per cent of that of the buffered virus.

At pH 4.5 and 3.0, a very small amount of virus passed through the candles at the beginning of filtration and until 70 cc. had filtered, after which the filtrate was noninfectious. Infectivity of the first 7 fractions of the filtrate at pH 4.5 was 0.108, 0.124, 0.093, 0.124, 0.054, 0.046, and 0.023 per cent of that in the nonfiltered. The first 7 fractions at pH 3.0 possessed 0.0032, 0.0016, 0.0032, 0.0024, 0.0032, 0.0024, and 0.0024 per cent, respectively, the infectivity of the nonfiltered.

All fractions were noninfectious when virus was filtered at pH 1.5. The nonfiltered virus remained highly infectious at this reaction.

The data in table 3, columns 2 and 3, were subjected to the analysis of variance, as developed by Fisher (10), in order to study biometrically the differences in estimated virus concentration of the various fractions. The analysis of variance consists of sorting out experimental variability and allocating it to different sources, leaving a residual portion ascribable to uncontrolled causes designated as error. The sums of squares form the basis for the measurement of variability. The mean value of the sums of squares ascribed to any source of variation (the variance or standard deviation squared) is obtained by dividing the sum of squares by the appropriate number of degrees of freedom, where the term degrees of freedom is used in the sense of independent comparisons. The significance of the difference between the different variances is determined by the z-test. A complete discussion of the analysis of variance is given by Fisher (10) and full details as to the arithmetical procedure are discussed by Fisher and Wishart (11).

In the data from fractionation of virus filtrates at pH 5.6 without buffers (Table 3, column 2), it is necessary to know if the estimate of variance due to filtration (various fractions) is significantly greater than that due to error. From the analysis (Table 4), it appears that the data are highly significant, since the observed value of z exceeds the calculated value of z at the 1 per cent point, indicating that differences as great as or greater than those due to chance would be found less than once in 100

TABLE 4.—*Analysis of variance of data presented in table 3, column 2*

Variation due to	Sum of squares	Degrees of freedom	Variance	Z
Tests	4,345,134,198.30	4		
Fractions	40,636,824,211.25	10	4,063,682,421.1	1.4017[a]
Error	9,851,924,413.29	40	246,298,110.3	
Total	54,853,882,822.85	54		

[a] = Exceeds the 1 per cent point.

times. By applying the difference required for significance (19,852) to the means, it is evident that the first filtered fraction is significantly different from the 2nd, 3rd, 4th, or 5th fraction, and also from the nonfiltered. Moreover, the 5th fraction is significantly different from all the others.

In order to determine whether the increased infectivity of virus filtered at pH 8.5 was biometrically significant, variance of the data was analyzed. Since the observed value of z (Table 5) for the analysis of variance of

TABLE 5.—*Analysis of variance of data presented in table 3, column 3*

Variation due to	Sum of squares	Degrees of freedom	Variance	Z
Tests	26,765,504,231	4		
Fractions	19,054,757,686	10	1,905,475,768.6	0.4975[a]
Error	28,173,592,769	40	704,339,819.2	
Total	73,993,854,686	54		

[a] = Exceeds the 5 per cent point.

these results exceeds the 5 per cent point, the data are significant. By applying the required difference for significance between any two means (33,570), it is to be noted that increased infectivity in all the filtered fractions was significantly different from the control or nonfiltered.

Correlation of Clogged Pores with Virus Retention

Clogged filter pores, as evidenced by the rate of flow of liquid, were correlated with the retention of virus during the fractional filtration experiments. The time required for each 10 cc. fraction to filter through at reactions of various pH values is given in table 6.

TABLE 6.—*Rate of filtration of tobacco-mosaic virus at reactions of various pH values through Berkefeld "W" candles*

Fraction of filtrate	Time to filter 10 cc.					
	Without buffer pH 5.6	pH 8.5	pH 5.6	pH 4.5	pH 3.0	pH 1.5
1st 10 cc.	15 sec.	20 sec.	20 sec.	25 sec.	27 sec.	32 sec.
2nd 10 cc.	16 "	20 "	30 "	30 "	37 "	95 "
3rd 10 cc.	20 "	20 "	40 "	75 "	50 "	5 min.
4th 10 cc.	29 "	20 "	55 "	172 "	70 "	10 "
5th 10 cc.	49 "	20 "	135 "	4.75 min.	90 "	15 "
6th 10 cc.	1.5 min.	20 "	3.5 min.	7.25 "	115 "	20 "
7th 10 cc.	3.66 "	20 "	6.75 "	10.0 "	147 "	30 "
8th 10 cc.	6.75 "	20 "	11.0 "	12.0 "	170 "	41 "
9th 10 cc.	17.5 "	20 "	17.5 "	14.5 "	3.16 min.	60 "
10th 10 cc.	30.0 "	20 "	23.5 "	17.0 "	3.75 "	62 "

These measurements show that the pores were not clogged at pH 8.5, as was shown by the constant rate of flow during filtration. At acid reactions the pores were clogged. With the 5th fraction at pH 5.6, they were not clogged to the extent of holding back virus, while afterwards they were reduced in size sufficiently to retain a part of the virus. This suggests a variation in the size of virus particles. At the other acid reactions the filters were impervious, or nearly so, at the beginning of filtration before the pores were clogged. In these cases, retention of virus by the filters was probably due to strong adsorption.

Fractional Filtration of Virus through Berkefeld "V" Candles

The quantity of virus passing through Berkefeld "V" candles in various fractions of the filtrate and the clogging of pores necessary to retain virus was determined by filtering virus at a 1×10^{-1} dilution in distilled water at pH 5.6. The results are given in table 7.

These data show that it required 70 cc. of the filtering suspension to clog the pores to the extent of retaining a part of the virus. At the beginning of filtration the first fraction of the filtrate was 38 per cent as infectious as the nonfiltered. After 20 cc. had filtered, the succeeding fractions were about as infectious as the nonfiltered juice until 70 cc. had filtered. After this the remaining fractions of the filtrate possessed from 6 to 30 per cent the infectivity of the nonfiltered juice.

Elution of Adsorbed Virus with 0.1 molar Disodium Phosphate

Virus adsorbed to the walls of the filter pores during filtration was eluted by filtering 100 cc. of 0.1 molar disodium phosphate solution through

TABLE 7.—*Virus concentration in successive 10 cc. fractions of Berkefeld "V" filtrate of tobacco-mosaic virus diluted* 1×10^{-1} *in distilled water*

Successive fractions of filtrate	Time to filter	Number of local lesions on 20 beans	Filtration
			per cent
1st 10 cc.	10 sec.	33,000	38.4
2nd 10 cc.	10 "	39,900	46.3
3rd 10 cc.	10 "	82,400	95.9
4th 10 cc.	10 "	93,500	108.8
5th 10 cc.	15 "	85,600	99.7
6th 10 cc.	30 "	122,400	140.1
7th 10 cc.	50 "	96,500	112.4
8th 10 cc.	3.33 min.	27,100	31.5
9th 10 cc.	10 "	5,000	5.8
10th 10 cc.	30 "	7,350	8.5
Unfiltered		85,866	
Check–H_2O		0	

the candles after the nonfilterable débris had been removed with a camel-hair brush. The filtrates were fractionated into 10 cc. portions in order to determine the rate of elution. The débris was suspended in 10 cc. of 0.1 molar disodium phosphate and the liquid filtered through cleaned candles. It was subsequently resuspended 9 times and filtered in order to determine the rate of virus elution from the nonfilterable materials in the original suspension. Estimated virus concentration of the various filtrates is given in table 8 as percentage elution of adsorbed virus.

TABLE 8.—*Elution of adsorbed virus from the capillaries of the filter and from the non-filterable débris with 0.1 molar phosphate buffer at pH 8.5*

Successive 10 cc. fractions of filtrate	Eluted virus	
	From filter	From débris
1st 10 cc.	85.34[a] ± 13.35	81.20 ± 9.10
2nd 10 cc.	9.16 ± 10.45	10.23 ± 5.73
3rd 10 cc.	2.68 ± 4.38	5.61 ± 5.39
4th 10 cc.	1.19 ± 1.01	1.18 ± 1.61
5th 10 cc.	0.77 ± 1.34	0.83 ± 1.55
6th 10 cc.	0.34 ± 1.29	0.31 ± 1.14
7th 10 cc.	0.21 ± 0.67	0.27 ± 1.21
8th 10 cc.	0.14 ± 0.40	0.17 ± 0.74
9th 10 cc.	0.09 ± 0.80	0.11 ± 1.61
10th 10 cc.	0.08 ± 0.87	0.09 ± 0.13

[a] = Per cent elution of adsorbed virus.

± = Probable error.

The quantity of virus eluted was proportional to the amount of virus adsorbed to the filter and to the nonfilterable débris. In the first 10 cc. of liquid, 85 and 81 per cent was released from the filter and from the débris, respectively, while in the 2nd 10 cc., 9 and 10 per cent was eluted. From 5 to 0.08 per cent was eluted in the remaining fractions.

The residue on the candles after washing the nonfilterable débris 10 times was suspended in 100 cc. (the original volume of virus filtered) of 0.1 molar disodium phosphate. The fact that the infectivity of this suspension to beans was very low indicated that a very small amount (0.0002 per cent of the original virus) of active virus remained with this material. The infectivity of a given amount of virus when added to the residue indicated the absence of any inhibiting substance.

Filtration of Virus through Positively Charged Filters

An attempt was made to determine the filterability of virus at controlled hydrogen-ion concentrations through plaster-of-Paris filters, which, according to Kramer (19), are positively charged.

Filters were prepared by molding plaster-of-Paris paste into discs the size of doubled Seitz E. K. filter pads. The paste consisted of commercial plaster-of-Paris to which 20 per cent of magnesium oxide had been added and an equal amount by weight of distilled water. After the discs had dried for a day, they were attached to Seitz filters and 20 cc. of virus (1×10^{-1} dilution) at pH 8.5 and 1.5 forced through under 100 cm. water pressure. After filtration, the discs used at pH 8.5 were washed by passing through 20 cc. of 0.1 molar phosphate buffer at pH 1.5, and those used at pH 1.5 were washed at pH 8.5. Infectivity of the filtrates and washings was determined by rubbing the samples on beans at pH 8.5.

The filtrates and washings were noninfectious. The fact that these filters were impervious to both basic and acid dyes indicates negative and positive areas in the discs. Apparently the positively charged surface adsorbed virus at pH 8.5 and Congo Red, while that negatively charged adsorbed virus at pH 1.5 and Victoria Blue. Negatively charged areas probably originated from impurities in the plaster-of-Paris or in the magnesium oxide.

Filterability of Virus in Suspension with Bacteria

Filterability and relative size of virus and *Bacillus prodigiosus* were determined in the following experiments. A mixture (100 cc.) of virus at 1×10^{-2} dilution and *B. prodigiosus* cells in heavy suspension was adjusted to pH 8.5 with 0.1 molar phosphate buffer and filtered through 6 different filters. The amount of virus in the filtered and nonfiltered suspensions was estimated by inoculation to beans and the number of bacteria per cc.

was determined by plating out a given quantity of the suspensions in nutrient agar. The results from these measurements are given in table 9.

TABLE 9.—*Filterability of tobacco-mosaic virus in a suspension of Bacillus prodigiosus at pH 8.5 in 0.1 molar phosphate buffer*

Filter	Diameter of filter pores	Bacteria per 1 cc.		Virus concentration[a]	
		Unfiltered	Filtrate	Unfiltered	Filtrate
Berkefeld "V"	10 μ	1,500,000	0	47,920	46,640
" "N"	7 μ	"	0	56,960	56,000
" "W"	4 μ	"	0	42,320	44,000
Seitz E. K.	...	"	0	20,480	24,640
Jena sintered glass	16 μ	"	4,000	20,760	30,240
Bechhold-Konig crucible	12 μ	"	3,000	23,920	20,000
Check		0		0	

[a] = Number of lesions on 20 Scotia beans.

Under the conditions of these experiments, filterability of virus was not affected by bacteria in the filtering suspension. Virus passed through all the filters, while bacteria passed only filters with maximum pores 12 μ or greater in diameter. The size of virus particles, according to these results, is considerably smaller than *B. prodigiosus*, which is 0.5 by 1 μ (Bergey, 6).

Ultrafiltration

Particle size of the virus was estimated by ultrafiltration through Bechhold's (1) acetic acid collodion membranes of various pore diameters.

The stock solution for the membranes consisted of 10 grams of soluble cotton (gun cotton) and 2.5 grams of anhydrous potassium carbonate dissolved in 100 grams of glacial acetic acid. Membranes of various pore sizes, as measured by Bechhold's (2) air-water method, were prepared from solutions containing different amounts of collodion. These solutions were obtained by diluting the stock collodion solution with glacial acetic acid. Membranes from solutions containing 10, 7, 4, and 2 per cent of collodion were prepared by coating the inner surface of Jena glass filters with the desired solution and then solidifying the collodion by submersion in distilled water. After washing the membranes for 2 hours, the maximum pore diameter of each membrane was calculated according to the formula, $r = \frac{2Q}{P}$, where r = radius of pores in cm.; Q = surface tension of water-air in dynes/cm. at temperature of measurement (72.5 dynes/cm. at 22° C.); and P = the pressure in dynes/sq. cm. required to force air through the membranes saturated with water. The pressure required to force air through the 10, 7, 4, and 2 per cent collodion membranes was 1,164–998,

1,209–914, 806–702, and 601–587 mm. of mercury, respectively. The maximum pore diameters, therefore, are 0.187–0.218, 0.181–0.238, 0.270–0.311, and 0.363–0.370 microns. The calibrated membranes were used for ultrafiltration with 700 mm. of mercury negative pressure. The Jena glass filters with adhering membranes were attached to suction flasks with Bailey funnels and 10 cc. of virus (1×10^{-1} dilution of a Berkefeld "W" filtrate) filtered through at pH 8.5 and 4.5. Virus held on the membranes was resuspended in 10 cc. of 0.1 molar disodium phosphate. Estimated virus concentration in the various suspensions is given in table 10.

TABLE 10.—*Ultrafiltration of tobacco-mosaic virus through Bechhold's collodion membranes of graded porosity*

Virus	Diameter of membrane pores (microns)	Reaction of medium	
		pH 5.4	pH 8.5
Control (unfiltered)		114,700[a] ± 6,947.35	173,638 ± 18,201.38
Ultrafiltrate	0.187–0.218	1[b]	0
"	0.181–0.238	0	0
"	0.270 0.311	0	24 ± 5.12
"	0.363–0.370	49 ± 24.28	1,296 ± 277.21
Residue	0.187–0.218	130,000 ± 20,235.00	157,600 ± 10,690.15
"	0.181–0.238	151,500 ± 13,017.85	171,000 ± 23,658.76
"	0.270–0.311	49,150 ± 25,113.95	81,000 ± 12,178.09
"	0.363–0.370	17,775 ± 1,838.01	52,700 ± 10,636.19
Check		0	0

[a] = Average number of lesions on 20 beans.
[b] = Probably a contamination.
± = Probable error.

It is apparent from these data that more virus passed through membranes at pH 8.5 than at pH 4.5. At pH 8.5 it just passed through membranes of 0.270 to 0.311 μ maximum pore diameter, but was retained by membranes with pore diameters of 0.187 to 0.218 and 0.181 to 0.238 μ. Assuming that the virus requires pores from 8 to 15 times its size in order to filter through, as Bechhold (3) found for bacteria, the size of the virus particles is estimated to be 0.018–0.038 μ (18–38 mμ[3]) in diameter. The

[3] m μ = millimicron, 10^{-9} meter.

residue recovered from the membranes impervious to virus was as infectious as the original virus sample, while that from the other membranes was about 50 per cent as infectious.

DISCUSSION

Filterability of tobacco-mosaic virus, like that of vaccine virus (Yaoi and Kasai, 34), is influenced by the reaction of the medium. The fact that the virus is completely filterable at pH 8.5 and nonfilterable at pH 1.5 indicates that the particles are negatively charged at pH 8.5 and positively charged at pH 1.5, since the filters (Berkefeld) are negatively charged throughout this pH range. The partial filterability at intermediate reactions suggests that only a part of the particles are negatively charged at these reactions. The electrical charge on the virus, as determined by cataphoresis, has been reported to be negative between the reactions of pH 4.0 and 9.0 by Takahashi and Rawlins (31), and between pH 5.3 and 8.5 by Olitsky and Hoffman (23), but the amount of virus negatively charged was not determined. According to cataphoretic measurements, the isoelectric point of at least a part of the virus is below pH 4.0. Data from the filtration experiments mentioned above suggest that the virus possesses an isoelectric range, the limits of which are below pH 8.5 and above pH 1.5. Associated amphoteric substances with different isoelectric points might impart an isoelectric range. Virus associated with a filterable substance negatively charged at a given hydrogen-ion concentration would be filterable, while that associated with nonfilterable substances or a filterable substance positively charged at the same reaction would be retained by the negatively charged filter. It was found that virus filterable through a negatively charged filter at a reaction of pH 8.5 was retained by a positively charged filter. Kramer (20) reported that virus (pH not given) was held back by filters that retained Congo Red and allowed Victoria Blue to pass through. The fact that the positively charged filters prepared for these studies contained negatively charged areas explains the retention of virus at pH 1.5. Retention of virus in acid media by Berkefeld candles was probably due to adsorption.

Nonfilterability of the virus at pH 1.5 may have resulted from precipitation at this reaction into aggregates larger than the filter pores. This seems improbable, since about 33 per cent of the retained virus was adsorbed within the pores at reactions of pH 5.6, 4.5, 3.0, and 1.5. If precipitation to this extent had occurred, the virus would have remained on the surface of the filter. A part of the virus, however, may have aggregated into nonfilterable particles. It is probable that the virus retained on the surface of the filter was adsorbed to nonfilterable particles in the virus suspension.

Virus infectivity of various fractions of the filtrates at pH 5.6 showed that adsorption of virus to the filter was strong at the beginning of filtration. When the candles were saturated (after filtering from 30 to 40 cc. through), virus was not held back, although clogging of pores continued. This is in agreement with Beijerinck's (5) statement that the first portions of the filtrate (pH not given) were less infectious than later fractions. Apparently after 50 cc. had passed through, clogging reduced the size of pore to the extent of retaining a part of the virus mechanically. The fact that coarse filters ("V") require more suspension for clogging than the fine filters ("W") indicates that pore size is the determining factor in retaining virus by clogged filters.

The adsorbing filter surface at pH 4.5 and 3.0 apparently was not saturated before the clogged pores retained virus. At pH 1.5 adsorption was sufficiently strong to prevent virus passing before the filters were clogged to any extent. At pH 8.5 there was no adsorption of virus or clogging of pores.

Relative elasticity of virus particles and bacteria may account for difference in filterability. Hatschek (14) reported that oil-emulsion particles under suitable pressure were changed in shape during filtration through pores smaller than their diameter, and upon emergence from the filters they assumed the original spherical shape. This explanation of virus filtration seems unwarranted, since the size of the particles determined by other methods approximates that by ultrafiltration. Bechhold and Schlesinger (4) reported the virus to be 50 mμ in diameter by ultracentrifugal analysis, and Waugh and Vinson (33) reported it 10 mμ in diameter by coefficient of diffusion analysis. By ultrafiltration the particle size has been estimated to be 15 mμ by MacClement and Smith (21). Duggar and Karrer (9) reported the virus particles to have a diameter of about 30 mμ. These estimates of the size of virus (10–50 mμ in diameter) are decidedly below the size of *Bacillus prodigiosus* (500 to 1000 mμ, Bergey, 6), but are in the order of magnitude of protein molecules, colloids, and particles of bacteriophage.

SUMMARY

When various dilutions of virus in distilled water were passed through Berkefeld "W" filters the infectivity of the filtrate as compared to the nonfiltered material was greatest in the highest dilutions.

Filterability of virus through Berkefeld "W" candles is determined by the reaction of the medium. At pH 8.5 virus is completely filterable, and at 1.5 nonfilterable. At intermediate pH values it is partially filterable.

Virus suspended in an acid medium is adsorbed to the Berkefeld filter at the beginning of filtration. After the filter surface is saturated with ad-

sorbed materials at pH 5.6, the filtrate is about as infectious as the non-filtered sample until the clogged pores retain most of the virus.

Virus adsorbed to the filters from an acid suspension is readily eluted in phosphate buffer at pH 8.5. About 80 per cent of the virus is released in 10 cc. of buffer.

Filtration of virus at pH 8.5 through Berkefeld "W" candles increased its infectivity 66 per cent.

Virus is separated from *Bacillus prodigiosus* by filtration through Berkefeld and Seitz E. K. filters.

The diameter of the virus particle is estimated to be 18 to 38 mμ.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, NEW JERSEY.

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EFFECT OF PHOSPHATE BUFFERS ON INFECTIVITY OF TOBACCO-MOSAIC VIRUS[1, 2]

H. H. THORNBERRY

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INTRODUCTION

Studies were made to determine whether various hydrogen-ion concentrations or the presence of salts enhance or depress the infectivity of tobacco-mosaic virus. Since differences in activity greater than about 25 per cent, as estimated by the local-lesion method, are significant, quantitative measurements on infectivity may be made with a fair degree of accuracy. The purpose of this paper is to report results of experiments that were designed to show the effect of different salts at different concentrations and various hyrogen-ion concentrations on infectivity of tobacco-mosaic virus.

REVIEW OF LITERATURE

It has been reported in the literature that a few substances completely inactivate the virus of tobacco mosaic but that many substances have no measurable effect upon it. Relatively few substances are known to increase virus infectivity. Allard (1) reported data that indicated increase of infectivity of tobacco-mosaic virus by sodium sulphate. An increase in apparent virus concentration in the presence of talc and norite was reported by Vinson and Petre (5). They also mention that primary potassium phosphate lowered infectivity, while 0.066 M phosphate buffer at pH 6.5 rendered the supernatant liquid from the lead-acetate-virus precipitate highly infectious. They state that alkaline reactions from pH 9.2 to 9.5 abruptly decreased virus activity. The addition of acid failed to restore the activity of virus that had been completely inactivated above pH 9.2. Lojkin and Vinson (2) reported that the enzymes employed in their studies had no effect upon the virus in fresh untreated tobacco juice, but that trypsin, pancreatin, and papain inactivated the virus of purified preparations. Stanley (4) found that trypsin at certain concentrations protected plants of *Phaseolus vulgaris* L. var. Early Golden Cluster and *Nicotiana glutinosa* L. against infection, while at lower concentrations, it partially inhibited infection. Virus in the virus-enzyme mixture became infective

[1] Presented as part of a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy: granted June 18, 1934.

[2] Published at the Expense of the Rockefeller Institute for Medical Research out of the regular order determined by the date of acceptance of the manuscript.

when the enzyme was destroyed by digestion with pepsin and by exposure to temperatures below the thermal death point of the virus.

METHODS

Virus infetivity was measured by the local-lesion method described by Price (3). Bean plants of the variety Scotia were used in all tests. Twelve days after planting seed, the upper surfaces of the primary leaves of 20 plants were inoculated by rubbing with a bandage-gauze pad moistened with 5 cc. of the virus sample to be tested. Leaves of check plants were similarly rubbed with a pad moistened with water. Because of variation in susceptibility of plants of different ages, all inoculations in a given experiment were made on beans that were planted on the same day and grown under favorable conditions in a greenhouse. Local lesions were counted 5 or 6 days after inoculation.

Johnson's tobacco-mosaic virus 1 was used throughout the studies. Infectious plant sap from macerated Turkish tobacco plants that had been infected for about a month was strained through bandage gauze and frozen immediately. Subsequently, on 2 occasions the liquid was thawed and refrozen. It was thawed and filtered through doubled bandage gauze before use in the experiments.

EXPERIMENTATION

Effect of Various Salts at Different Concentrations on Infectivity

For a preliminary survey of the effect of salts on infectivity, virus was treated with 24 different salts and 2 phosphate buffer solutions at pH 8.0 and 6.5 before inoculation to beans. The following chemicals were used: trisodium, disodium, dipotassium, diammonium, monopotassium, and monosodium phosphate; sodium carbonate; sodium bicarbonate; sodium and ammonium citrate; sodium tetraborate; boric acid; potassium fluoride, chloride, bromide, and iodide; sodium, ammonium, calcium, and aluminum chloride; and sodium, ammonium, magnesium, and aluminum sulphate. Each was employed at 0.01, 0.05, 0.1, 0.5, and 1 molar concentrations. When solubility of the salt permitted, 2, 3, 4, 5, and 6 molar concentrations were used also. Virus at a dilution of 1×10^{-2} in the salt solutions of various concentrations was allowed to stand 1 hour at room temperature. The inoculations were made without changing the salt concentration or reaction of the virus suspension. All plants were washed in running tap water after inoculation to remove the excess salt from the inoculated leaves. Control plants were inoculated with virus at 1×10^{-2} dilution in distilled water.

The results showed that certain concentrations of the dibasic phosphate salts increased virus infectivity. Ammonium, sodium, and potassium di-

basic phosphate at 0.1 molar concentration (pH 7.2, 8.5, and 8.5, respectively) increased infectivity 229, 353, and 411 per cent, respectively. Trisodium phosphate (pH 11.2) inactivated the virus, while sodium carbonate (pH 10.5) greatly reduced its infectivity, but in no case completely inactivated it. The acid salts, aluminum chloride and sulphate, at all concentrations tested greatly reduced or completely inhibited infection. At 0.1 molar concentration these salts reduced infectivity 91 and 99 per cent, respectively. There was no significant effect upon infectivity by the other salts up to 0.1 molar concentration. Above 1.0 molar concentration all salts, soluble to that extent, reduced infectivity. These data show that the dibasic phosphate salts increase the infectivity of virus to beans, and that 0.1 molar concentration is optimum. There was no evidence that valency of the anion or cation influenced infectivity. The effect of monobasic and dibasic phosphate salts and 2 phosphate buffers at pH 8.0 and 6.5 on the number of lesions produced is shown in figure 1.

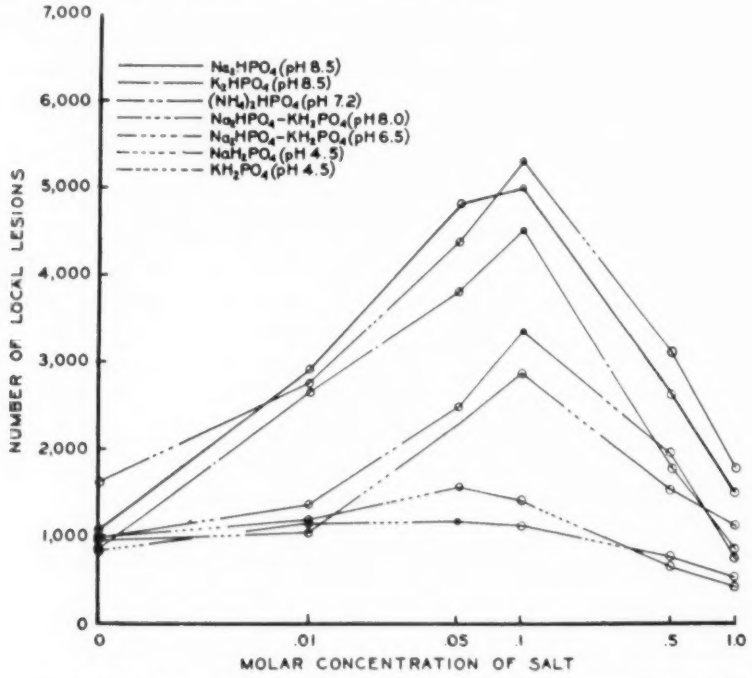

FIG. 1. Graphs showing the effect of 5 phosphate salts and 2 phosphate buffers (pH 8.0 and 6.5) in varying concentrations upon the infectivity of tobacco-mosaic virus at 1×10^{-2} dilution.

Effect of Disodium and Dipotassium Phosphate upon Infection

The effect of disodium and dipotassium phosphate at 0.01, 0.05, 0.1, 0.5, and 1.0 molar concentrations upon infectivity of the virus at dilutions

TABLE 1.—*The effect of disodium and dipotassium orthophosphate upon the infectivity of tobacco-mosaic virus at various dilutions*

Salt	Virus dilution	Control no salt	Molar salt concentration					Increase in infectivity at 0.1 M salt concentration
			0.01	0.05	0.1	0.5	1.0	
								per cent
Na_2HPO_4 (pH 8.5)	1×10^{-0}	6328[a] ± 286.6	7657 ± 296.7	7951 ± 282.6	9341 ± 336.5	7750 ± 243.4	3522 ± 232.7	47
	1×10^{-1}	3491 ± 447.1	5648 ± 551.0	7133 ± 490.3	9065 ± 983.4	6753 ± 480.9	3832 ± 324.4	160
	1×10^{-2}	1098 ± 81.6	2884 ± 509.9	4778 ± 350.7	5028 ± 257.6	2672 ± 254.9	1482 ± 192.2	358
	1×10^{-3}	352 ± 42.4	834 ± 275.1	1007 ± 120.7	1769 ± 369.8	1008 ± 138.9	454 ± 45.1	403
(pH 8.5) K_2HPO_4	1×10^{-0}	3549 ± 151.0	4837 ± 390.5	6145 ± 747.3	8487 ± 76.2	5350 ± 583.4	3627 ± 292.0	139
	1×10^{-1}	1463 ± 294.0	2982 ± 248.8	4955 ± 549.7	6250 ± 603.6	3241 ± 120.7	2169 ± 273.1	320
	1×10^{-2}	885 ± 164.5	2701 ± 126.1	3812 ± 146.3	4522 ± 380.4	1800 ± 29.0	860 ± 101.1	411
	1×10^{-3}	343 ± 11.4	1343 ± 238.7	1823 ± 66.1	2085 ± 707.5	976 ± 18.8	172 ± 6.7	508

[a] = Average number of lesions on 20 beans.
± = Probable error.

of 1×10^{-0}, 1×10^{-1}, 1×10^{-2}, and 1×10^{-3} was determined. The salt-virus mixture was allowed to stand 1 hour before inoculation. Control plants were inoculated with virus at comparable dilutions in distilled water. The results are given in table 1.

The data show that infectivity in all cases was increased by the salts, up to 0.5 molar concentration. In 1.0 molar concentration the virus was as infectious as that in the control. The greatest increase in all cases was at 0.1 molar concentration of salt. There was no significant difference between the effect of the disodium and dipotassium phosphate. Concentration of 0.1 molar disodium of dipotassium phosphate, therefore, is optimum for infection on Scotia beans with virus at dilutions up to 1×10^{-3}.

Effect of Phosphate Buffers at Various Hydrogen-ion Concentrations upon Infection and Virus Activity

Effect of hydrogen-ion concentration upon infection was determined by inoculating the samples of virus of a 1×10^{-2} dilution at reactions of various pH values. Effect of the buffers upon virus activity was measured by exposing virus to various hydrogen-ion concentrations for 1 hour and then adjusting the reaction to pH 8.5 before inoculation. Phosphate buffers at 0.1 molar concentration of salt were used to maintain the desired

TABLE 2.—*The effect of pH upon infection and activity of tobacco-mosaic virus*

pH	Infection at given pH value	Activity of virus after exposure to given pH
Control (5.6)	895[a] ± 60.77	2740 ± 291.31
1.5	239 ± 15.37	2730 ± 186.97
2.0	510 ± 84.04	2450 ± 180.22
3.0	863 ± 24.21	2593 ± 263.72
4.0	1183 ± 98.61	2407 ± 309.39
4.5	1208 ± 90.58	2487 ± 188.59
5.0	1348 ± 140.29	2443 ± 295.49
6.0	1738 ± 136.31	2419 ± 314.11
6.8	2281 ± 294.01	2643 ± 237.49
7.0	2935 ± 80.60	2743 ± 279.58
7.2	3154 ± 137.59	2737 ± 225.82
8.0	3504 ± 50.85	2733 ± 202.61
8.5	3098 ± 81.34	2677 ± 304.94
9.0	1525 ± 41.68	2477 ± 127.41
10.0	1010 ± 69.60	1951 ± 462.16
10.3	919 ± 116.68	1225 ± 85.99
10.5	645 ± 180.83	677 ± 185.55
10.6	264 ± 42.20	124 ± 36.35
10.7	17 ± 1.14	15 ± .87
10.8	0	0
11.0	0	0
11.2	0	0
Check	0	0

± = Probable error.
[a] = Average number of lesions on 20 beans.

hydrogen-ion concentration between pH 1.5 and 11.2. Control plants were inoculated with virus diluted 1×10^{-2} in distilled water. The results are given in table 2.

These data show that reactions from pH 7.0 to 8.5 are optimum for infection on Scotia beans. In more acid or more alkaline media the virus was decidedly less infectious. Inoculations for the measurement of virus infectivity or concentration, therefore, should be carried out at a reaction between pH 7.0 and 8.5. When the virus was adjusted to pH 8.5 before inoculation to beans, there was no appreciable difference in the infectivity of the samples that had been exposed for 1 hour between pH 1.5 and 9.0. Reactions above pH 9.0 reduced infectivity, while those from pH 10.8 to 11.2 inactivated the virus. It appears from these data that virus activity is not changed in 1 hour at room temperature between pH 1.5 and 9.0.

Effect of 0.1 Molar Disodium Phosphate on Dilution Capacity of Virus

Virus infectivity at dilutions from 1×10^{-0} to 1×10^{-8} in 0.1 molar disodium phosphate was compared with virus at comparable dilutions in distilled water. The suspensions were allowed to stand for 1 hour at room temperature before inoculation to Scotia beans. Results of the experiments are given in table 3.

TABLE 3.—*Effect of 0.1 molar disodium phosphate upon the measurement of virus concentration by the local-lesion method on beans*

Dilution value	Virus diluted in	
	Distilled water	0.1 M Na_2HPO_4
1×10^{-0}	7416[a] ± 536.9	9813 ± 664.3
1×10^{-1}	4333 ± 358.8	8306 ± 386.4
1×10^{-2}	1489 ± 80.9	4892 ± 242.1
1×10^{-3}	485.4 ± 60.0	1815.6 ± 191.5
1×10^{-4}	58.2 ± 24.2	567.0 ± 81.6
1×10^{-5}	7.0 ± 1.3	37.0 ± 3.9
1×10^{-6}	0.6 ± .51	3.8 ± .45
1×10^{-7}	0.0	0.4 ± .51
1×10^{-8}	0.0	0.0
Check	0.0	0.0

[a] = Average number of lesions on 20 beans.
± = Probable error.

These results show that 0.1 molar disodium phosphate increased the infectivity of virus at all dilutions that were infectious. At 1×10^{-7} dilution the sample containing salt was infectious, while that without salt was

noninfectious. In no case was infection obtained from the virus at a 1×10^{-8} dilution. The number of lesions produced by virus at 1×10^{-4} to 1×10^{-7} in 0.1 molar disodium phosphate was proportional to the dilution value.

Rate of Virus Inactivation in Alkaline Phosphate Buffers

The rate of virus inactivation in alkaline phosphate buffers was determined. Inactivation was measured by exposing for varying periods of time a 1×10^{-2} dilution of virus to phosphate buffers at pH 9.0, 10, 10.5, 10.6, 10.7, 10.8, 11.0, and 11.2. At the end of the desired exposure, each virus suspension was adjusted to pH 8.5 before inoculation to beans. Control plants were inoculated with a 1×10^{-2} dilution of virus in distilled water. The results are given in table 4.

TABLE 4.—*Inactivation of tobacco-mosaic virus by alkali at room temperature. The reaction was adjusted to pH 8.5 before inoculation to beans*

Virus dilution 1×10^{-2} in 0.1 M phosphate buffer	Time of exposure								
	5 mins.	10 mins.	15 mins.	30 mins.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
pH 9.0					2660[a]	2920	1760	3300	3120
pH 10.0					3300	2780	3220	2080	2420
pH 10.5					1680	1880	1220	68	192
pH 10.6					52	4	4	0	0
pH 10.7					4	0	0	0	0
pH 10.8	368	132	260	36	0				
pH 11.0	20	16	0	0	0				
pH 11.2	0	0	0	0	0				
Control in H_2O									
pH 5.6	2500	2230	2680	2835	2540	2340	2600	3160	2720
Check	0	0	0	0	0	0	0	0	0

[a] = Number of local lesions on 20 beans.

The table shows that virus was inactivated within 5 minutes at pH 11.2, and within 15 minutes at pH 11.0. At pH 10.5 inactivation was slight within 1 hour, and incomplete in 6 hours. Inactivation was complete at this dilution within 1, 2, and 4 hours at pH 10.8, 10.7, and 10.6, respectively. There was little change in virus activity at pH 9.0 or 10.0.

Rate of Virus Inactivation in Acid Phosphate Buffers

The rate of virus inactivation in acid buffers was determined. For these experiments, virus at 1×10^{-2} dilution was exposed for 1, 2, 3, 4, and 6 hours in solutions at various acidities. At the end of the exposure period

the samples were adjusted to pH 8.5 before inoculation to beans. Phosphate buffers more acid than pH 1.5 (acidity of 0.1 molar H_3PO_4) were obtained by adding HCl to 0.1 molar H_3PO_4. The acidity of these buffers is expressed in normality of HCl. Aluminum sulphate at 0.05 and 0.1 molar concentration was included. The control consisted of a comparable dilution of virus in distilled water. The results are given in table 5.

TABLE 5.—*Inactivation of tobacco-mosaic virus by acid at room temperature. The reaction was adjusted to pH 8.5 before inoculation to beans*

Virus dilution 1×10^{-1} in acid media	Time of exposure				
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
in 0.1 M. phosphate buffer at pH 2.0	3120[a]	2600	3140	2760	3140
in 0.1 M. phosphate buffer at pH 1.5	2550	3040	3000	2940	3060
in 0.1 M. H_3PO_4 and 0.01 N HCl	3380	2800	2460	2600	2740
in 0.1 M. H_3PO_4 and 0.1 N HCl	2300	2300	2200	2980	1560
in 0.1 M. H_3PO_4 and 0.5 N HCl	0	0	0	0	0
in 0.1 M. H_3PO_4 and 1.0 N HCl	0	0	0	0	0
in 0.1 M. $Al_2(SO_4)_3$	3540	2940	3020	2860	3200
in 0.05 M. "	2780	2900	3200	3240	3060
Control — in water pH 5.6	3440	1840	1900	1880	3020
Check	0	0	0	0	0

[a] = Number of local lesions on 20 beans.

It is evident from these data that 0.5 and 1.0 N HCl inactivates the virus in 1 hour at room temperature. Apparently slight inactivation occurred in 0.1 N HCl in 6 hours, but none in 0.01 N HCl or at pH 1.5 or 2.0. Aluminum sulphate had no effect upon virus activity when the reaction was adjusted to pH 8.5 before inoculation.

DISCUSSION

Infectivity of tobacco-mosaic virus was found to be influenced by salt and hydrogen-ion concentration. At 0.1 molar concentration, nearly all the salts tested increased infectivity to some extent, while concentrations above 1.0 molar reduced the amount of infection. This reduction in infectivity may have resulted from the precipitation of virus into fewer infective particles or from the action of high salt concentrations upon the inoculated plants. Reduction in the presence of aluminum sulphate was, no doubt, due to the acidity of the solution (about pH .15) and not to the presence of the salts, since the virus possessed its original infectivity when the reaction was adjusted to pH 8.5 before inoculation.

Dibasic phosphate salts in solution at 0.1 molar concentration (pH 7.2 and 8.5) greatly increased infectivity, while monobasic phosphate salts

(pH 4.5) increased infectivity very little as compared to the control, which was at pH 6.5. It appears that the amount of enhancement in infectivity is determined by an interaction of hydrogen-ion concentration and salt concentration.

The influence of salt and hydrogen-ion concentration upon infectivity of the virus may be either an effect on the virus or on the host plant, or both. At present, methods are not available for determining the effect upon susceptibility of the host plant. Apparently, virus is not inactivated in 1 hour at acidities as great as pH 1.5. If inactivation occurs at these reactions, virus activity is quantitatively reversible, since original infectivity of the virus is restored upon adjusting the reaction to pH 8.5 before inoculation. The reduction in infectivity above pH 9.0 and complete inhibition of infection at pH 10.6 in 1 hour is, no doubt, virus inactivation. Complete inactivation in acid or alkali is not reversible. This agrees in general with the result on alkaline inactivation reported by Vinson and Petre (5).

In order to maintain favorable conditions for estimating virus concentration or activity, the reaction of the suspension should be adjusted between pH 7.0 and 8.5 in 0.1 molar phosphate buffers previous to inoculation to beans. Due to the difficulty of counting lesions in excess of 300 per leaf, concentrated samples should be diluted before inoculation.

SUMMARY

Valency of the anion or cation of the salts tested had no measurable effect upon infectivity of tobacco-mosaic virus.

Dibasic phosphate salts at 0.1 molar concentration greatly enhanced virus infectivity.

Optimum reaction for infection is from pH 7.0 to 8.5.

Virus was not inactivated in 1 hour between pH 1.5 and 9.0. At pH 10.6 complete inactivation resulted in 4 hours, and at pH 11.2 in 5 minutes. In 0.5 molar HCl inactivation was complete in 1 hour.

The presence of aluminum sulphate greatly reduced infection, but when the reaction was adjusted to pH 8.5 the original virus activity was restored.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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THE INITIATION OF DOWNY MILDEW OF TOBACCO IN NORTH CAROLINA IN 1934[1]

LAWRENCE F. DIXON, RUTH A. MCLEAN, AND FREDERICK A. WOLF

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INTRODUCTION

Evidence was presented in a previous report[2] that downy mildew of tobacco is initiated as the result of employing the same areas, in successive years, as sites for tobacco seed beds. This evidence was based (1) upon the observation that oospores of the pathogen are present within the tissues of affected leaves of tobacco seedlings, and (2) upon the observation that the disease may appear several weeks earlier in seed beds located on the sites of old beds than on those located on new situations. The fact also was established,[2] as determined by the results of trapping experiments, that sporangia of the organism are carried by currents of air. It also may be recalled that downy mildew spread rapidly throughout the flue-cured tobacco districts of Georgia, South Carolina, North Carolina, and Virginia in 1931, the season of its first appearance, and that it subsequently encroached upon the tobacco-growing areas of Maryland, western North Carolina, Tennessee, and Pennsylvania. The reasonable assumptions have been made that air-borne sporangia were the inocula for the introduction of the pathogen into these areas, that the fungus hibernated in situations where the climate was mild, and that it was reintroduced each season into the more northerly areas. A study of the initiation of downy mildew in North Carolina, in 1934, was, therefore, conducted to test these assumptions, and thus to learn more about the hibernation of the fungus and the source of inoculum for primary infection of tobacco in this State.

METHODS

Apparently no one has been able, as yet, to acquire sufficient knowledge regarding the period of dormacy of the oospores of the downy-mildew fungus and of the conditions that are favorable for germination of the oospores, to utilize such information in a study of the yearly initiation of the disease. To date, our studies along this line have given only meagre

[1] A cooperative investigation by the Departments of Botany and Chemistry, Duke University. The writers wish to express their gratitude for their cooperation to Dr. P. M. Gross, and to the several agricultural teachers and farm demonstration agents and interested growers for their cooperation.

[2] Wolf, Frederick A., L. F. Dixon, Ruth McLean, and F. R. Darkis. Downy mildew of tobacco. Phytopath. 24: 337–363. 1934.

and erratic results; consequently, epiphytological methods have been employed in the present investigation. Accordingly, two procedures have been followed.

(a) Stations were established at which daily observations could be made to determine the date of first appearance of the disease in beds located on the sites of old beds in comparison with those on new situations. These stations (Fig. 1) were located near McDonald, N. C. (Robeson County), and Durham, N. C. (Durham County).

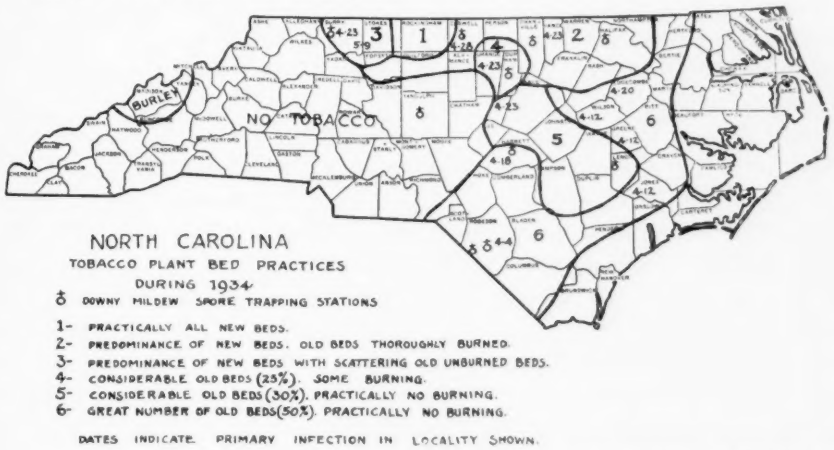
BURLEY
NO TOBACCO
NORTH CAROLINA
TOBACCO PLANT BED PRACTICES
DURING 1934
DOWNY MILDEW SPORE TRAPPING STATIONS
1- PRACTICALLY ALL NEW BEDS.
2- PREDOMINANCE OF NEW BEDS. OLD BEDS THOROUGHLY BURNED.
3- PREDOMINANCE OF NEW BEDS WITH SCATTERING OLD UNBURNED BEDS.
4- CONSIDERABLE OLD BEDS (25%). SOME BURNING.
5- CONSIDERABLE OLD BEDS (50%). PRACTICALLY NO BURNING.
6- GREAT NUMBER OF OLD BEDS (50%). PRACTICALLY NO BURNING.
DATES INDICATE PRIMARY INFECTION IN LOCALITY SHOWN.

FIG. 1. Map of North Carolina showing the areas in which primary infections were noted, as indicated by the dates; the stations at which spore traps were exposed; and regional practices in seed-bed care.

In making the examinations, the cover was first removed from the bed and then the entire bed was subjected to a systematic and careful scrutiny, as shown by the fact that in one instance a single infected plant was found in a bed with an area of 300 square yards, in another, 3 plants in a bed 200 square yards in area. Spread of inoculum and contamination of the beds was guarded against by attention to such sanitary precautions as frequent changing of shoes and cleansing of hands. It is felt that the frequent examination of the beds did not serve to introduce the downy mildew fungus into any of them, because the disease did not, in any case, appear in the beds that were closely watched prior to its appearance in adjacent, similar, unmolested seed beds.

In order to supplement the observations at these stations, surveys of beds, both of those on old sites and on new sites, were made at certain other points in North Carolina; namely, near Clinton (Sampson County), Snow Hill (Greene County), Wilson (Wilson County), Lillington (Harnett County), Rocky Mount (Edgecombe County), Oxford (Granville County),

Yanceyville (Caswell County), Reidsville (Rockingham County), and Mount Airy (Surry County). The surveys were begun prior to the outbreak of downy mildew in these areas, and examinations were made at frequent intervals until the disease became generally prevalent. The examination and reexamination, both of beds located on old sites and of those on new sites, were made with equally painstaking care. Beds on both types of sites were examined on the same day.

(b) Stations were established in North Carolina to determine, by means of trapping experiments, the earliest occurrence of air-borne sporangia, as related to the earliest occurrence of the disease in that section. These stations were located near Rowland (Robeson County), McDonald (Robeson County), La Grange (Lenoir County), Lillington (Harnett County), Littleton (Halifax County), Farmer (Randolph County), Yanceyville (Caswell County), Dobson (Surry County), Durham (Durham County), and Oxford (Granville County), (Fig. 1). The spore traps consisted of glass plates, 13.3 sq. cm. in area, coated with glycerine and placed 30 inches above the ground level. Four plates, oriented to face each of the cardinal points of the compass, were exposed every 48 hours in each place except near McDonald, Durham, and Oxford. At these places, 8, 10, and 12–24 plates, respectively, were exposed each 2-day period. A total of 1,357 plates were exposed, in which task the teachers of agriculture in the different locations generously cooperated. Examinations were made by first washing off the glycerine and adhering materials, centrifuging the washings, and then examining the sediment microscopically.

RESULTS

(*a*) *With incidence of downy mildew in beds on old sites and those on new sites:* An idea of practices in one of the areas under observation, with reference to the employment of new and old bed sites and of their relative proximity, can be gained from figure 2, that represents the area near McDonald, N. C.

The growth of tobacco seedlings throughout the State was delayed in 1934 by cold weather. The seedlings were, therefore, smaller than they were at a corresponding date in late March or early April of 1933.

The search for primary infections in 1934 was begun when the tobacco seedlings were less than $\frac{1}{4}$ inch in diameter, and diseased plants were detected when the seedlings had attained a plant spread of $\frac{1}{4}$ to $\frac{1}{2}$ inch. In the majority of instances primary infections were found when sporangia were present on relatively few (1–25) plants in beds 200 to 300 square yards in area. The leaves in contact with the soil were found to be involved first, and at a time when these leaves had not yet become yellow, although yellowing is commonly regarded as the earliest symptom of downy mildew.

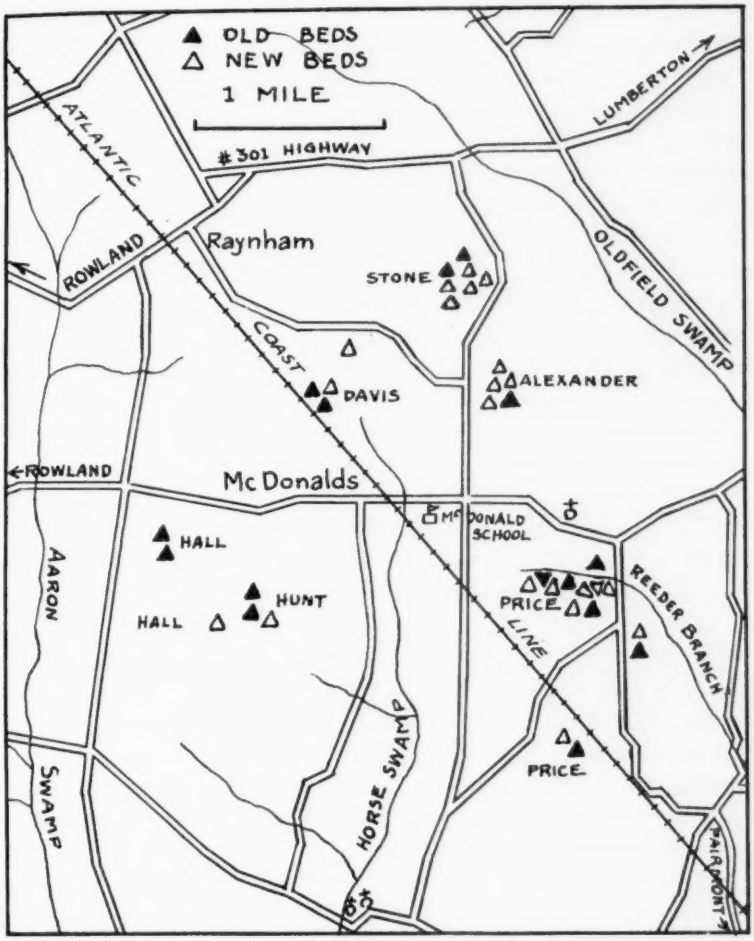

FIG. 2. Map of the environs of McDonald, N. C., showing the distribution of seed beds.

In 1933, the disease was first found somewhat earlier than in 1934, and on seedlings that were considerably larger. The disease had spread, when first seen, to the extent of involving the seedlings in an area a yard or more in diameter. In the light of the present season's observations, there is reason to believe that the disease must have been present, in 1933, 10 to 14 days prior to its discovery. The infections first observed in 1933, therefore, were presumably secondary infections initiated by sporangia from former primary infections in those beds.

Certain data relating to the occurrence of downy mildew in beds located on old sites and in near-by beds on new sites have been assembled in table 1.

The essential features from these data are (1) the distribution of the beds over the flue-cured tobacco-growing area of the State, (2) the approximately equal number and area of beds on the two types of sites, (3) the time of the first occurrence of downy mildew in each locality in beds on each type of site, and (4) the earliest date on which sporangia were entrapped in the air in each locality.

The most striking fact established by this series of observations is that infections occurred earliest, in all cases, in beds located on the sites of beds that were diseased in 1933. No exception to this was noted. Not all primary infections were evident on the same day in any given locality. In Robeson County, for example, an interval of six days occurred between the time of finding the first primary infected bed and the last one, and in Surry County this interval was of 16 days' duration. The fact that the period of primary infections extended in the mountainous Surry County area, over a longer period of time than in the coastal plain region, is explained by the widely differing physiographic features of this mountainous county. To the extent that these features varied locally within Surry County, there was a corresponding variation in the advance of the season, growth of natural vegetation and dates of occurrence of primary infections.

The next important feature is the fact that sporangia were not entrapped in any area until after the downy-mildew fungus was sporulating in old beds in that area. It seems entirely reasonable to infer from these observations, therefore, that the primary infections originated from inocula that had hibernated in these beds. That such inocula consisted of oospores is the logical assumption, as an exhaustive search for overwintering sucker-bearing plants was made and only two such plants were found, neither of which contained hibernating mycelium.

In 1934 downy mildew was first noted in North Carolina on April 4, near McDonald. It was first noted in South Carolina in a bed on an old site near Hemmingway, on the previous day. Attention also should be called to the fact that the initial appearance of the disease in Georgia was about a month earlier, and that it did not become wide-spread in Georgia until about two weeks after it was first found in North Carolina. The disease spread slowly because of the unusually dry, sunny weather prevalent during the downy-mildew season in the tobacco-growing areas surveyed. This slow spread facilitated the distinction between primary and secondary infections.

Another generalization may be made regarding the time of occurrence of primary outbreaks to the northward and westward from McDonald, N. C., as shown in table 1. As the distance from this point increased, the date of the first occurrence of the disease was progressively later, and there was approximately three weeks' difference in time between the first outbreak

TABLE 1.—*Incidence of downy mildew in beds on old sites and in those on new sites in various sections of North Carolina in 1934*

Locality (County)	Beds on old sites					Date when sporangia were first trapped	Beds on new sites			
	No. of beds examined	Area of beds examined (sq. yds.)	No. of beds showing primary infections	Area of primarily infected beds (sq. yds.)	Period in which primary infections were first evident		No. of beds examined	Area of beds examined	No. of beds infected during primary infection period	Period in which infections were first evident
Robeson	30	2,470	9	760	April 4–9	April 7	25	3,280	0	April 16–28
Greene	10	2,350	1	300	April 12	April 20	2	350	0	April 20–28
Wilson	14	2,600	7	1,650	April 12–18	.....	7	1,250	0	April 20–28
Edgecombe	2	150	1	60	April 20	.....	2	260	0	May 2–10
Harnett	11	2,600	7	1,950	April 18–23	None	12	2,925	0	May 2–10
Durham	14	1,870	5	500	April 23–24	May 7	20	3,498	0	May 4–11
Granville	6	1,400	2	750	April 23	April 30	5	2,500	0	May 4–11
Sampson	6	700	0	0	.....	.....	11	2,100	0	May 5–10
Caswell	5	920	1	220	April 23	April 24	8	1,330	0	May 10–17
Rockingham	3	500	0	0	.....	.....	7	550	0	May 12–19
Surry	5	850	4	700	April 23–May 9	May 8	9	1,600	0	May 12
Total	106	16,010	37	6,890	April 4–May 9	April 7–May 8	108	19,643	0	April 16–May 19

in the southeastern portion of the flue-cured area and that in the northwestern one. The differences correspond closely with the seasonal differences between these sections. The earliest outbreaks occurred at the time when flowering dogwood, *Cornus florida*, came into blossom. The disease did not reach epiphytotic proportions, in 1934, in southeastern North Carolina until about 3 weeks later than in 1933, but was only 7 to 10 days later in the central Piedmont section.

It also may be noted that 37 of the 106 beds located on the sites of old beds, or 35 per cent, showed primary infection. This proportion is believed to be representative of conditions throughout the State. At first glance, this proportion may not seem important, but if it be borne in mind that none of the 108 beds located on new sites became diseased until subsequent to the occurrence of the disease in some of the near-by beds on old sites, the importance of primary infection of beds on old sites will become apparent. Certain of the remaining 69 beds located on the sites of old beds became diseased earlier than certain of the beds on new sites, others, coincident with them, and others, after the disease had developed in certain of the beds on new sites. The fact that downy mildew does not occur in all beds sown on old sites prior to its occurrence in beds sown on new sites has led to confusion of opinion among growers and investigators concerning this matter of the influence of site on the occurrence of primary infections. It might be expected that the fungus should survive the winter and appear first in all beds on old sites, instead of in a portion of them. Undoubtedly, the character of the site and the treatment given the seed bed in the interval between crops, as pointed out later in this report, influences the chances of survival of oospores. The fact also should be stressed, as has been stated, that primary infections in beds on old sites do not involve all plants in these beds simultaneously, but a few only. Evidently, a relatively small number of oospores, even under the most favorable natural conditions, survive the winter in any given old bed site. The fact that the remaining 65 per cent of the beds on old sites became infected during the same period as those beds on new sites, and exhibited the appearance of secondary infections, led to the presumption that sporangia were the source of inoculum in these old beds.

The use of old bed sites rather than new ones may be the factor that determines whether the grower will be able to secure a sufficient number of seedlings at the time most propitious for transplanting. Downy mildew in primarily infected beds may cause the death of a large number of the seedlings and inhibit the growth of those that survive. This situation is represented in figure 3, that shows two beds of the same age in the same neighborhood, as they appeared on May 10, 1934. The one on the old site, shown above, was found to be primarily infected on April 23, and the disease soon

FIG. 3. Seed beds near Durham, N. C., as they appeared on May 10, 1934. The upper one was located on the site of an old bed; the lower one on a new site.

involved all of the seedlings. There was no evidence of disease in the other one on the new site, until May 11. Those that survived in the former did not recover until too late to be used, whereas two lots of seedlings had been drawn for transplanting from the latter before May 11.

(b) *With air-borne sporangia:* The differences in time existing between the primary sporulation in beds sown on old sites and the first occurrence of air-borne sporangia in the vicinity of such beds (Table 1) indicates that the northward progression of downy mildew, in 1934, can not be satisfactorily accounted for by assuming that air-borne sporangia have been introduced from distantly removed southern areas. The data seem rather to indicate that the sporangia borne on primarily infected seedlings serve as inoculum for secondary infections in other seed beds in that same locality. The differences in dates of occurrence of primary infections at the different stations to the northward of McDonald, N. C., correspond with the seasonal differences at these stations, as has been shown. It would appear, therefore, that the wave-like northward progression of the disease might more reasonably be attributed to differences in the time of ocurrence of primary infections, in the several localities, rather than to sporangia that may have been transported to these localities.

Spread of sporangia for short distances has been known to have been accomplished by the agency of man. Evidence, from our observations during the past season, indicates that in some cases the inoculum was spread by the grower. Indiscriminate passage from infected areas to noninfected ones, during weeding, or from one bed to another, resulted in the carriage of sporangia on the hands and clothing of the grower and in subsequent infection. It is also possible that disease-free beds located on old sites, or those on new sites, may become contaminated in this manner.

(c) *With seed bed practices:* The season's observations have emphasized the existence of a variety of practices as to methods of preparation and care of the seed beds and of handling the seed bed sites after the seedlings have been transplanted.

The tobacco-growing sections of the State have been classified as shown in figure 1, according to the prevalent practice in regard to seed-bed care and preparation. While variations occur within nearly all of the sections, a given practice usually was sufficiently widespread to justify a generalized classification. The evidence from observations made in 1933 and 1934 indicates that the number and distribution of old beds in any given locality largely determine the rate of spread of the disease, and, in so doing, modifies the seriousness of the outbreak in that locality. The general situation as to the relationship of the handling of old bed sites and the incidence of primary infection is given in the data compiled in table 2.

TABLE 2.—*Seed bed practices as related to the incidence of primary infection*

Treatment of beds	No. of beds on old sites	No. of beds showing primary infection	No. of beds infected simultaneously with beds on new sites	Primary infections in each class
				(*Per cent*)
No infection during 1933	3	0	3	.....
Burned prior to 1934 sowing	20	4	16	20.0
Not burned prior to 1934 sowing	62	33	29	53.2
Thoroughly burned (4 cords of wood per 100 sq. yds. of bed)	11	1	10	9.1
Slightly burned (brush piles, corn stalks, few pine slabs)	9	3	6	33.3
Abandoned to plants remaining after transplanting. *Not burned* prior to 1934 sowing	25	13	12	52.0
Abandoned to plants remaining after transplanting. *Burned* prior to 1934 sowing	15	2	13	13.3
Cultivated in corn or beans after transplanting seedlings. *Not burned* prior to 1934 sowing	5	2	3	40.0
Cropped with cowpeas between plantings. *Not burned* prior to 1934 sowing	3	1	3	33.3
Covered with wheat straw between plantings. *Not burned* prior to 1934 sowing	10	9	1	90.0
Plowed immediately after transplanting, but not sown in any crop. *Not burned* prior to 1934 sowing	4	4	0	100.0

The data employed in table 2 deal with 85 of the 106 seed beds examined, and present on the sites occupied by beds in 1933. The remaining 21 beds, on old sites, have not been used because their treatment was different from any of the classifications made in table 2. Even though the number of cases in some of the classes is small, the results are believed to be significant. Bed sites that have been "burned" prior to sowing are less likely to show primary infection than those not burned, but this practice cannot be depended upon to completely destroy all inoculum. The covering of the beds with a thick layer of straw immediately after transplanting has been completed and, allowing it to remain undisturbed until time for re-

seeding, is conducive to the preservation of inoculum. The plowing under of the seedlings remaining after transplanting, and reseeding in the following spring, seems also to favor the protection of the inoculum in the soil. This is especially true in beds located in damp, shaded situations.

DISCUSSION

The downy-mildew fungus is known to attack the seed pods of mature plants in the field in Australia,[3] and the seed from such pods have been shown, by microscopic examination, to be invaded by the mycelium of the pathogen. Infection of the seed pods of field-grown plants has not been noted, however, in North Carolina. For this reason infected seed are not believed to constitute a means for the survival of the fungus from season to season here nor for its introduction into seed beds.

Occasional tobacco plants are known to survive the winter in this State. As previously noted, two such plants bearing living suckers were found in the spring of 1934, in spite of the severity of the preceding winter. Both were sectioned and stained but no evidence was found of hibernating mycelium within their tissues. Suckers affected with downy mildew have been found, however, in mid-winter, in southern Georgia. It appears improbable from the observations of the past three years in North Carolina that the survival of the pathogen on old plants constitutes an important factor in its hibernation within the State.

SUMMARY

A study has been made, by epiphytological methods, of the sources of inoculum for the primary infections of downy mildew of tobacco in North Carolina.

The pathogen overwinters in this State, presumably in the oospore stage. There is no evidence that it survives on plants that may have escaped being killed by cold nor that it is seed-borne.

Infections have been found to develop earlier in seed beds located on the sites occupied by seed beds the previous year than on those sown on new sites. These infections were found to occur prior to the time that sporangia could be entrapped from the air in the same locality. The northward march of the disease is attributed to seasonal influences that modify the time of occurrence of primary infections in various localities.

Primary infections were found in 35 per cent of the seed beds sown on the sites of old beds. The downy-mildew fungus was sporulating in beds on old sites from 7 to 19 days prior to the development of the disease in any near-by seed beds located on new sites.

[3] Angell, H. R., and A. V. Hill. Downy mildew (blue mould) of tobacco in Australia. Counc. Sci. Ind. Res. Australia Bull. 65. 1932.

Seed beds sown on the sites of old beds are concluded to constitute primary centers of infection.

Air-borne sporangia or sporangia carried by the grower account for secondary infections.

None of the practices employed in North Carolina with seed beds located on old sites can be depended upon to destroy all of the inoculum in the soil.

The avoidance of utilizing the same site for seed beds in succeeding years is, therefore, of major importance in the prevention of downy mildew of tobacco in the State.

A VIRUS OF CRUCIFERS AND OTHER HOSTS[1]

ISMÉ A. HOGGAN AND JAMES JOHNSON

(Accepted for publication September 22, 1934)

Mosaic of cruciferous plants has been recorded from widely separated districts of the United States (1, 2, 3, 4) and from Europe (5), though whether one or more viruses are concerned can not be determined with certainty from the descriptions published. In the present note a fuller description is given of a crucifer mosaic virus known to affect cabbage, mustard, turnip, and horse-radish. This virus was first brought to the writers' notice in the winter of 1933 by Dr. R. H. Larson of the Department of Plant Pathology, University of Wisconsin, who called attention to some turnip plants showing a conspicuous mottling of the leaves, suggestive of mosaic infection. The plants were among a number being grown for seed production in a cool greenhouse (about 60° F.) and were believed to have contracted the disease in the field during the preceding summer.

By means of artificial inoculation with extracted juice a virus was recovered from two of these plants, which proved to be infectious to various cruciferous and other host species. This virus was transmissible mechanically by plant extract and more readily by aphids, *Myzus persicae* Sulz. and *Brevicoryne brassicae* L. The symptoms on cruciferous hosts appeared to vary considerably according to the temperature and the age of the plants. In general, at about 70–80° F., cabbage, *Brassica oleracea* L. var. *capitata* L., seedlings showed a mild mottling or chlorotic spotting of the leaves (Fig. 1, A, B). Seedlings of mustard, *B. nigra* Koch, and turnip, *B. rapa* L., developed a severe necrosis and yellowing of the foliage, and, later, if the plants survived, a distinct mottling, sometimes accompanied by savoying of the lamina. Leaves and root cuttings of horse-radish, *Armoracia rusticana* Gaertn., believed to be mosaic-diseased, were received in May, 1934, from Kenneth J. Kadow, Illinois Agricultural Experiment Station, Urbana, Illinois, and these proved to contain a similar virus. Plants grown from the root-cuttings showed distinct vein-clearing and a mottling or chlorotic spotting of the foliage.

More striking, however, were the symptoms caused by this virus on tobacco, *Nicotiana tabacum* L. var. Connecticut Havana No. 38, which developed conspicuous brown necrotic lesions on the leaves at points of infection (Fig. 1, C, D, and Fig. 2). These lesions first became visible in

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

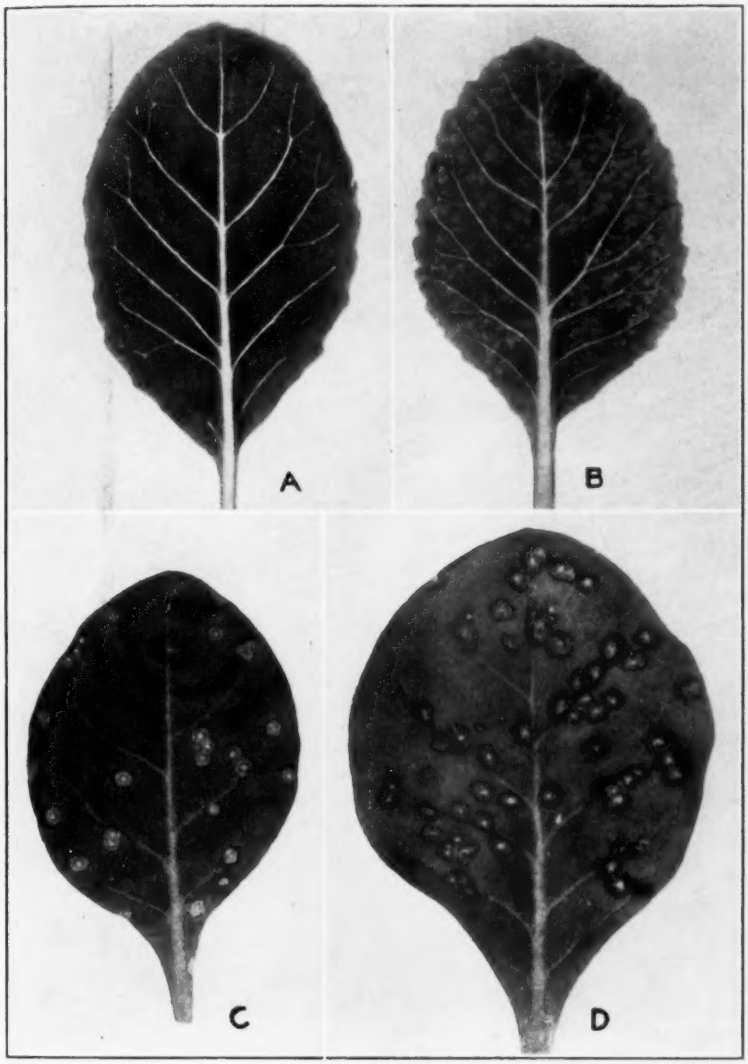

FIG. 1. A. Leaf of healthy cabbage seedling. B. Leaf of cabbage seedling showing mottling caused by the crucifer mosaic virus. C. and D. Leaves of tobacco showing local lesions caused by the crucifer mosaic virus. C. Young lesions produced by *Myzus persicae* from infected cabbage. D. Slightly older lesions produced by rubbing-inoculation from diseased horse-radish.

about 3 or 4 days, and enlarged rapidly up to 5 mm. or more in diameter, usually showing a darker necrotic band at the edge. They sometimes spread a short distance along a vein, but there was no systemic invasion

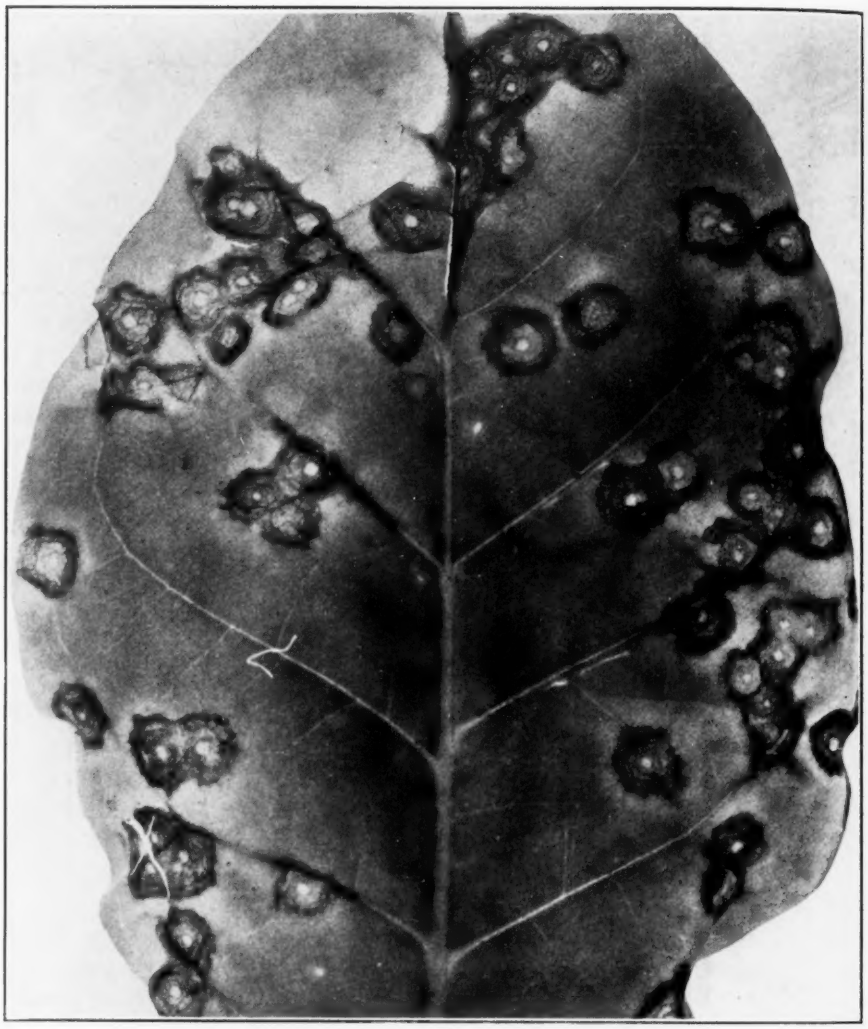

FIG. 2. Old local lesions of the crucifer mosaic virus on tobacco. Approximately $\times\frac{3}{2}$.

of this host. Similar local lesions occurred on the hybrid, *N. tabacum* × *N. glutinosa*, though on *N. glutinosa* only mild chlorotic spots developed, with occasional faintly necrotic rings. Faint chlorotic local lesions developed also on red currant tomato, *Lycopersicon pimpinellifolium* Dunal. On spinach, *Spinacia oleracea* L. var. Bloomsdale, the virus caused systemic infection in the form of a conspicuous yellow spotting and, later, a fine, distinct mottling.

The properties of the virus in extract from cabbage and horse-radish were studied by means of inoculation to tobacco. Each sample of extract,

after treatment, was inoculated by the rubbing method to 2 or 3 leaves of each of 5 tobacco plants, and the number of lesions developing was counted a week or more later. The results of several different tests are presented in summarized form in table 1. It was noted that nontreated extracts from

TABLE 1.—*Properties of the crucifer mosaic virus as determined by the number of lesions formed by rubbing inoculation of extract on tobacco*

Thermal death-point			Tolerance to dilution			Longevity *in vitro*		
Temp. °C. (10 min.)	No. of trials	No. of lesions	Dilution	No. of trials	No. of lesions	Age of extract in hrs. (20–22° C.)	No. of trials	No. of lesions
Nonheated	5	1756	None	5	927	0	4	1219
50	2	115	1 to 10	5	232	12	2	105
52	1	6	1 to 100	5	43	24	4	84
53	3	9	1 to 1000	5	10	36	2	20
54	2	0	1 to 10,000	5	2	48	3	5
55	3	0	1 to 100,000	5	0	72	3	0
Noninoculated controls	5	0	Noninoculated controls	5	0	Noninoculated controls	4	0

horse-radish gave considerably more lesions on tobacco than similar extracts from cabbage, although the point of inactivation of the virus in these tests did not appear to be influenced by the host. The thermal death point for a 10-minute treatment was found to be 54° C. and the tolerance to dilution 1 to 1,000 (in one test 1 to 10,000). The longevity *in vitro* at about 20–22° C., usually lay between 24 and 48 hours, though in one test slight infection was obtained after 48 hours. The longevity *in vitro* was always less than 3 days at the temperature specified.

In a limited study, no inclusion bodies have been observed in association with the disease.

The necrotic local lesions on tobacco are of particular interest in relation to their possible occurrence on this host under field conditions. Tobacco is subject to a variety of leaf spots, the origin of some of which still remains obscure. It is not unlikely that tobacco grown near cruciferous crops or weeds may occasionally become infected with this crucifer virus through the agency of aphids. If so, the connection might not ordinarily be easy to determine, for the virus is not readily recovered from affected tobacco leaves; hence, such leaf spots might be attributed to malnutrition or some other nonparasitic cause.

SUMMARY

A description is given of a crucifer mosaic virus transmissible to various cruciferous and other hosts. Characteristic symptoms are produced on tobacco, consisting of conspicuous necrotic local lesions without systemic infection. It is not known whether this virus is identical with the crucifer mosaic viruses previously reported by other investigators.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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FACTORS THAT INFLUENCE THE FORMATION AND DEVELOPMENT OF MYCORRHIZAL ASSOCIATIONS IN CITRUS ROOTS[1]

H. S. Reed and Thérèse Frémont

(Accepted for publication November 9, 1934)

The conditions for physiological equilibrium between the cells of the Citrus root and their endophytic mycorrhiza appear to depend in large measure upon the chemical and physical nature of the soil and upon the seasonal activity of the tree.

This paper contains the results of studies on this problem in continuation of work initiated at this institution by Rayner.[2]

The material utilized by Rayner and by the writers was collected from orange, *Citrus sinensis*, trees growing on plots on which experiments with fertilizers are being conducted by the Department of Orchard Management of the Citrus Experiment Station. Certain plots have been fertilized annually by definite schedules for the past 7 years; others have received no fertilizers during that time. We have examined roots from: (a) plots that received cover crops and stable manure, (b) plots that received no fertilizers, and (c) plots that received sodium nitrate.

Roots growing in soils that received annually applications of cover crops and stable manure appeared to develop a definite resistance to the invading fungus.[3] There was comparatively little intracellular mycelium in roots from such soils, the hyphae being restricted to short branches that entered the root cells. Once within the root cells, these branches developed ramifications that resemble the "arbuscules" of Gallaud and other investigators.[4] The entrance of the fungus appears to provoke an active response on the part of the root cell, as a result of which the cytoplasm becomes spongy, envelops the arbuscules, and is rich in mitochondria (Fig. 1, A). These changes do not extend to all the cytoplasmic mass, but are confined to the region of the cell containing the invading hyphae, whose contents show modifications similar to those exhibited by the host cytoplasm.

The nature of the cytological changes supports the idea that an active proteolysis operates to digest, first, the intracellular mycelium, then the adjacent cytoplasm of the root cell.

[1] Paper No. 295, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Rayner, M. C. Mycorrhiza in the genus Citrus. Nature (London) **131**: 399–400. 1933.

[3] Reed, H. S., and Th. Frémont. Sur les réactions des cellules des racines de Citrus à l'infection par les mycorrhizes. Compt. Rend. Acad. Sci. (Paris) **199**: 84–87. 1934.

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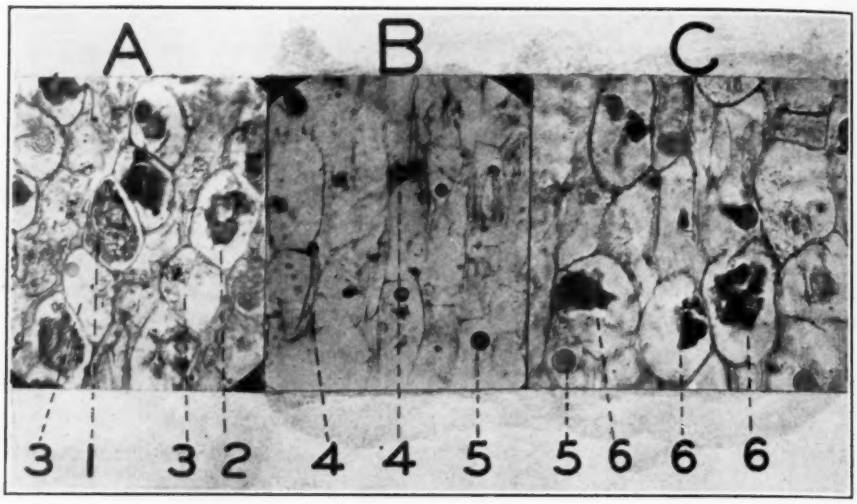

FIG. 1. Photomicrographs of infected orange-root cells showing specific responses to the soil treatments. A. Root from tree that received cover crops and stable manure. 1, cell whose spongy cytoplasm envelops the arbuscule; 2, early stage of digestion; 3, later stage of digestion. B. Root from tree that received no fertilizers. 4, intracellular mycelium of the endophyte; 5, phenolic precipitate. C. Root from tree that received sodium nitrate. 5, phenolic precipitate; 6, hypertrophied cells containing dense masses of endophyte and disorganized cytoplasm.

Roots growing in soils that had received no fertilizers during the preceding 7 years had little power to resist invasion or to digest the intracellular hyphae. In them the mycelium of the fungus seemed to grow as a true parasite. The number of the cells invaded by the fungus was relatively small, the endophyte living principally as intercellular mycelium (Fig. 1, B). The branches that it sent into the cells of the cortical parenchyma were short and sparingly ramified, differing little in their histological characters from the intercellular mycelium. The ends of these branches were often in contact with the nuclei of the cells, as in numerous cases of parasitic Peronosporaceae.

The infection of the root cells, moreover, never provoked an active digestive response on the part of the host, as in the case of roots that received suitable applications of organic and inorganic fertilizers.

The roots of trees that received sodium nitrate as the only fertilizer showed none of the "mycorrhiza cells" in which there was infection followed by digestion. The cortical cells were characterized by their abnormally large size and by an exaggerated reaction to the penetration of the endophytic fungus (Fig. 1, C). Their granular contents absorbed stains with such avidity that it was difficult to recognize anything resembling the

invading hypha, which was the primary cause of the modification of the cell, except where the cytoplasm of the host cell was extremely vacuolated. The trees under discussion that received all their fertilizer in the form of sodium nitrate were unthrifty and suffered severely from the disease known as mottle-leaf. The root cells contained phenolic precipitates which indicated profound disturbances in their metabolism. No evidence of a beneficial association with a fungus could be found.

The relations between the root cells and the fungus also varied with the season when the samples were taken and with the corresponding physiological activity of the tree. The infection of roots occurs principally when the tree is actively growing in the spring months. After growth ceases the mycorrhiza cells gradually disappear. Roots taken from the soil fertilized with suitable forms of organic nitrogen contained an abundance of infected cells only during the period of active, vernal growth. Roots taken from the unfertilized trees, however, contained the endophytic fungus at all seasons of the year. In them, as we have said, the mycelium was intercellular and the reaction of the plant very weak.

In presenting this report we wish to emphasize the importance of the mycorrhizal association as a factor in the metabolism of Citrus-root cells. The relations between the root cell and the endophytic fungus contribute to an understanding of the responses of the trees to nitrogenous fertilizers, and to the conditions favorable for infection by the endophyte.

Citrus Experiment Station,
Riverside, California.

PHYTOPATHOLOGICAL NOTES

Breeding millet resistant to smut in North China.[1]—Millet, *Chaetochloa italica*[2] Scribn., is second only to wheat as a food crop in North China. About 22,814,440 acres are devoted to its cultivation, and the annual production amounts to approximately 28,675,548,000 pounds.[3]

As reported by Porter *et al.*,[4, 6] kernel smut, caused by *Ustilago crameri* Körn., is a very important disease of millet in China. In the summer of 1932 the disease was very destructive in the vicinity of Kaifeng, Honan, the amount being as high as 25 per cent in at least one field.

Kernel smut can be materially reduced by the use of clean seed and proper seed treatment.[4, 6] Moreover, Porter *et al.*[5] also stated that resistant strains may be readily selected. Since the original resistant strains are no longer available, the present writers have resumed the task of developing resistant varieties of millet to meet the local need.

Altogether, 1,430 head selections were made in the Fall of 1932. Of these 1,273 heads were selected from fields in the vicinity of Kaifeng, while the other 157 heads were selected from farmers' varieties collected from different localities of the province of Honan. Originally, these varieties were tested primarily for high yielding ability and were under test for 4 years. An unnamed variety (No. 48) was secured for checks from Mr. Y. H. Pi, of the cooperative Agricultural Experiment Station of the University of Nanking, and the Southern Baptist Mission at Kaifeng, Honan. This variety yields well and possesses desirable agronomic characters, but its reaction to certain diseases it not known.

The inoculum was obtained from a field in the vicinity of the College of Agriculture, Honan University, Kaifeng, in the fall of 1932. The seeds were thoroughly dusted with the smut spores just before planting.

The planting was made on June 11 to 12, 1933. Each selection was planted to a 15 Chinese-foot[7] row, with a foot between each row. The seed

[1] The authors are greatly indebted to Dr. E. C. Stakman and Dr. J. J. Christensen, of the University of Minnesota, U. S. A., for reading critically the manuscript.

[2] AAMODT, O. S. Varietal trials, physiologic specialization, and breeding spring wheats for resistance to *Tilletia tritici* and *T. levis*. Canadian Jour. of Research, 5: 501–528. 1931.

[3] CHANG, C. C. An estimate of China's farm and crops. 1932.

[4] PORTER, R. H. Seed disinfectants for the control of kernel smut of foxtail millet. Proc. 3rd Pan-Pac. Cong., Tokio, pp. 2103–2107. 1926.

[5] PORTER, R. H., H. K. CHEN, and T. F. YU. Smut resistance in millet. Phytopath. 20: 915–916. 1930.

[6] PORTER, R. H., T. F. YU, and H. K. CHEN. The effect of seed disinfectants on smut and on yield of millet. Phytopath. 18: 911–919. 1928.

[7] A Chinese foot is equivalent to $\frac{1}{3}$ of a meter.

was sown at the rate of 3.5 grams seeds per row. Every tenth row was planted to a check in order to determine variability, if any, due to soil heterogenity, and to make agronomic comparisons. The experiment was not replicated on account of insufficient seeds.

At maturity each row was harvested separately and the percentage of infected heads determined.

To facilitate a detailed examination of the data, the selections were grouped into seven classes on the basis of their susceptibility. Percentages of infection are classified according to Aamodt's scheme, with slight modifications. (Table 1.)[8]

TABLE 1.—*The reaction of 1,430 head selections of millet to kernel smut, Ustilago crameri*

Percentage of smut	Type of reaction	No. of selection	Percentage of selection
0	I	192	13.4
0.1– 5	HR	119	8.3
6.0– 15	R	198	13.8
16.0– 30	MR	379	26.5
31.0– 45	MS	289	20.2
46.0– 60	S	183	12.8
61.0–100	HS	70	4.9

There were striking differences in the susceptibility among the selections. The percentage of infection ranged from 0 to 87 per cent. Although most of the selections developed more than 5 per cent of smut, 192 remained free, while approximately 80 of the selections were more susceptible than the variety used as a control (8.5 per cent). Some of the smut-free selections also possess desirable agronomic characters. Apparently, the varieties of millet commonly grown by farmers in the province of Honan are in heterozygous condition with respect to smut resistance. The data substantiate previous conclusions that selection within a variety is a promising means of isolating desirable smut-resistant strains of millet already adapted to a local community.—CHIH TU and H. W. LI, Honan University, Kaifeng, China.

[8] 0 per cent infection = Immune = I
.1 – 5 per cent infection = Highly resistant = HR
6 – 15 per cent infection = Resistant = R
16 – 30 per cent infection = Moderately resistant = MR
31 – 45 per cent infection = Moderately susceptible = MS
46 – 60 per cent infection = Susceptible = S
61 – 100 per cent infection = Highly susceptible = HS

An Example of Spread of Veinbanding from Potatoes to Tobacco.[1]—Veinbanding, described by Valleau[2] as one of the components of the potato rugose-mosaic complex, often is present in tobacco on the Experiment Station farm at Lexington, Kentucky, and in certain other tobacco plantings. Valleau and Johnson[3] stated that veinbanding is almost never found in tobacco unless it is near potatoes. This virosis usually appears in tobacco prior to handling operations, which indicates that it is transmitted from some other plant by insects. Smith[4] showed that veinbanding (his "y" virus) is readily transmitted from potatoes to tobacco by *Myzus persicae* Sulz. This species is less common on potatoes in Kentucky than the pink and green aphid, *Microsiphum solanifolii* Ashm. It has not been shown that aphids transmit veinbanding from potatoes to tobacco, in the field, but winged forms are not uncommon on tobacco. Even though they do not readily colonize, they may feed enough to cause infection.

In 1934 there was an exceptional opportunity for observing the spread of veinbanding from potatoes to tobacco on the Station farm. Five plots of White Burley tobacco were set the last week in May, near 2 plots of Irish Cobbler potatoes, one of which was planted in the spring and the other about the middle of July. A diagram of the tobacco and potato plots is given in figure 1 and descriptions of the tobacco plots in table 1.

TABLE 1.—*Descriptions of tobacco plots*

Plot No.	No. of Rows	No. of Plants	Position of Rows
1	196	16,722	At right angles to potatoes
2	41	2,477	Parallel to potatoes
3	41	2,375	Parallel to potatoes
4	53	2,812	Parallel to potatoes
5	102	10,210	At right angles to potatoes

The numbers in figure 1 are percentages of veinbanding in groups of 5 rows each of tobacco. The groups of plants in plots 1 and 5 are further divided into thirds, represented by the lines drawn lengthwise of the plots, to better show the decreasing amount of veinbanding as the distance from potatoes becomes greater. In plot 1, in 90 rows opposite potatoes, in the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

[2] Valleau, W. D. The viruses concerned in rugose mosaic of Irish Cobbler potatoes and the weed-host problem. Phytopath. **20**: 135. 1930.

[3] Valleau, W. D., and E. M. Johnson. The relation of some tobacco viruses to potato degeneration. Ky. Agr. Expt. Sta. Bul. 309. 1930.

[4] Smith, K. M. On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators. Proceedings Roy. Soc. Ser. B. **109**: 251–267. 1931.

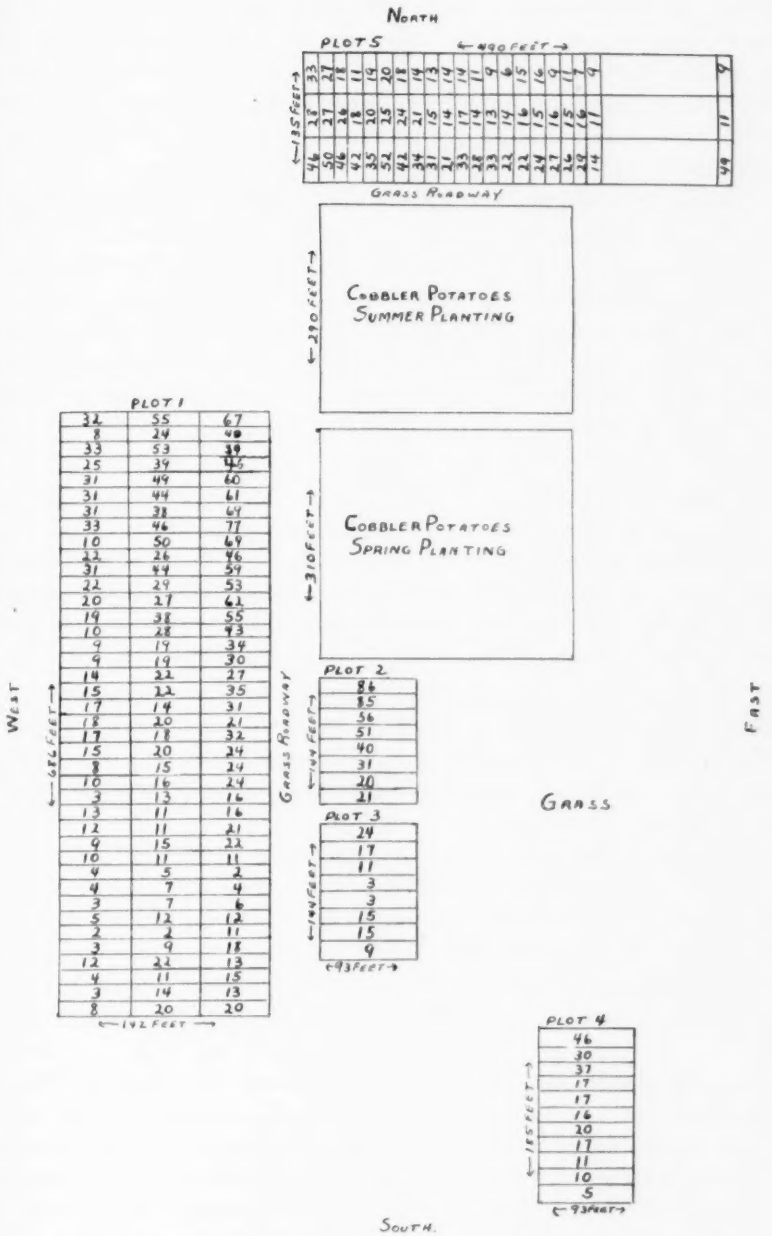

FIG. 1. Percentages of veinbanding in tobacco plots near potatoes.

third nearest potatoes, the middle third, and the third farthest away, 53, 36, and 21 per cent of the plants, respectively, were affected with veinbanding. In the remaining 106 rows, not directly opposite potatoes, the percentages of veinbanding for the corresponding thirds were 18, 13, and 10. In plot 5 the percentages for the corresponding thirds of the plot were 35, 18, and 14.

Observations were made in 4 other tobacco plots on the Experiment Station farm. In one plot of 414 plants, 60 feet from potatoes, 66 per cent of the plants had veinbanding while another plot, 200 feet from the same potatoes, had 19 per cent. Two other isolated plots had 7 and 3 per cent, respectively, the one with the smaller percentage being the greater distance from potatoes. The percentages in all plots show a rapid decrease of veinbanding as the distance from potatoes increases.—E. M. JOHNSON, Kentucky Agricultural Experiment Station.

A root rot of Chinese elms.—A serious root rot of Chinese elms, *Ulmus pumila* L. and *U. parvifolia* Jacq., has recently been discovered on nursery stock grown throughout the Great Plains region. From the diseased roots the fungus *Chalaropsis thielavioides* Peyronel has been consistently isolated. This fungus was originally described by Peyronel[1] as the cause of a disease of lupine and has recently been described by Hammond[2] as the cause of a graft disease of walnuts. Spores of this fungus produce typical rot when placed in Chinese elm roots that have been cut or broken. Fusaria and bacteria are frequently present in the diseased tissue and nematodes have been found consistently associated with *C. thielavioides* in naturally infected roots.

The original infection probably takes place in the seed beds and may spread rapidly in storage or during transit. A grayish white mold-like fungus growth, which appears at injuries or broken places on the roots, is the first observable symptom. The outer root tissues become dark brown to black and are broken down to a slimy mass as the rot advances. Infected plants in the nursery row usually show only the black decayed areas on the roots.

The typical microconidia and macroconidia of *Chalaropsis thielavioides* are produced in abundance, both on decayed roots and in pure cultures Fig. 1). The size of the hyaline to light brown cylindrical microconidia varies considerably in length and thickness but fits in general the dimensions given by Peyronel for his species. These usually are produced from

[1] Peyronel, B. Una nuova malattia del lupino. Staz. Sper. Agr. Ital. **49**: 583–596. Tav. II to VI. 1916.

[2] Hammond, J. B. A graft disease of walnuts caused by a species of *Chalaropsis*. (Abstract) Trans. Brit. Mycol. Soc. **19**: 158–159. 1935.

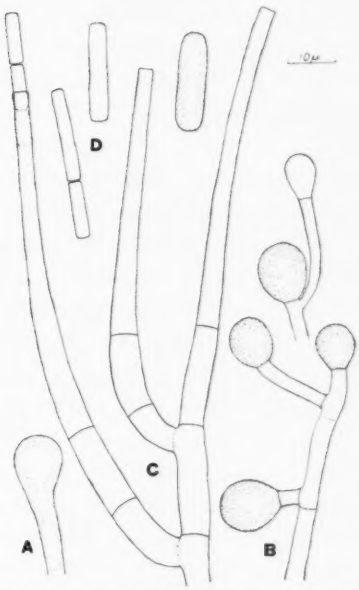

FIG. 1. Conidia and conidiophores of *Chalaropsis thielavioides* from elm. A and B. Macroconidia in various stages of development. C. A group of conidiophores bearing microconidia. D. Microconidia showing variation in size and manner of endogenous formation.

long conidiophores that are hyaline or light brown. The macroconidia are dark, broadly, ovoid to spherical, and borne on short, hyaline conidiophores. Their dimensions also agree with those given by Peyronel. Both types of conidia are very similar to the condidia of *Ceratostomella fimbriata* (E. & H.) Elliott,[3] which always produces a perfect stage; but no perithecial or sexual structures have yet been found for the elm fungus.

Inoculations have definitely proven the pathogenicity of the fungus to *Ulmus pumila* and *U. parvifolia*. Cross inoculations to a number of other tree species have been mostly negative, but there are some indications that the fungus may cause some trouble to several different species, especially as a parasite of cuttings.

Control measures are still in the experimental stage, but it is probable that a chemical dip treatment will be the best means of preventing spread in storage. The fungus appears to live over in the soil. The establishment of new seed beds or the sterilization of old beds with steam or formaldehyde would probably likewise aid in the control of the disease. The elimination of storage should also prevent the spread and rapid development of the fungus.

[3] Andrus, C. F., and Harter, L. L. 1933. Morphology of reproduction in *Ceratostomella fimbriata*. Jour. Agr. Res. **46**: 1059–1078. 6 figs., 2 plates. 1933.

The origin of the disease in the Great Plains region is unknown—HOWARD LAMB, ERNEST WRIGHT, and ROSS W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Emergency Conservation Work and Shelter Belt Project.

Mites on Potatoes.—In the issue of PHYTOPATHOLOGY for July, 1933,[1] is described a potato disease the cause of which is a mite, now definitely identified as *Tarsonemus latus* Banks. The description of the disease as originally published needs slight revision. It now appears that the mites probably were not responsible for the injury to plants in the field. At any rate, no mite injury occurred in the same field in either of the 2 succeeding years, although mites were found on plants in nearby greenhouses. The brittleness of stems and particularly of stolons should not be ascribed to the mites alone. Aside from these slight changes, the injury to potato plants has been found in further observations to be substantially as stated.

In addition to the solanaceous host plants mentioned in the first article, similar severe injury has been observed on beans, *Phaseolus vulgaris*, and on young apple trees, *Pyrus malus*, in greenhouse culture.

Some mild objection has been made (acroamatic) to the implication that it is necessary to graft plants or mutilate leaves in order to transfer the mites. The mites, of course, get about very easily and there is even a suggestion that they may be transferred occasionally and inadvertently by winged creatures. The criticism is valid enough. The explanation is simple. The original outbreak of mites occurred on hybrid plants and for some time it was assumed that this was either another virus disease or else a reaction of these particular hybrid seedlings to acronecrosis or to acropetal necrosis, both of which had become prevalent in the cultures at about the same time and all of which were being studied by the same methods.—DONALD REDDICK.

[1] Reddick, Donald. A potato disease. Phytopath. 23: 622–624. 1933.

BOOK REVIEW

Gitman, L., and E. Boychenko. *Spravochnik po bolezniam novykh loobianykh kul'tur.* [*Manual of diseases of new bast fiber plants.*] 124 pp. 86 figs. Lenin Academy of Agricultural Sciences, Moscow, U. S. S. R. 1934. (In Russian, with English preface and summary.)

Because of the serious drawback caused by fungous parasites to the successful cultivation of the various new bast-fiber plants now extensively grown in the Soviet Union, the Division of Plant Protection of the New Bast Fiber Research Institute of the Lenin Academy of Agricultural Sciences undertook the task of systematizing and codifying all available data bearing on this subject. The authors of this conveniently arranged ready reference brought together in concise form a mass of information hitherto scattered in many different publications or still remaining in the form of unpublished notes or incomplete diagnoses. The object of the little volume as stated in the introduction by Professor N. A. Naumov is to "facilitate the identification of [bast fiber] diseases according to their external manifestation and to the microscopic peculiarities of the pathogen." The manual consists of two major parts. The first part comprises a general discussion of the morbid anatomy of the different parts of the bast fiber plants due to the invasion of bacteria and fungi. In the second part detailed information is given regarding each known malady, and is arranged in a schematic, more or less tabular form. The host plants are arranged in taxonomic order, the plant pathogens are listed in alphabetical order, and the diseases are grouped according to plant parts affected. Disease symptoms and etiology are carefully recorded and literature references are fairly abundant. The many illustrations should prove very helpful.—M. N. Levine, University Farm, St. Paul, Minnesota.